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# FACTORS INFLUENCING THE REPRODUCTIVE CYCLE IN THE CHIMPANZEE; THE PERIOD OF ADOLESCENT STERILITY AND RELATED PROBLEMS

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DURING a comparison of the reproductive cycles displayed by a group of ovariectomized chimpanzees with those shown prior to the operations (Young, Fish and Dorfman, 1942), our attention was attracted to certain changes and fluctuations in the character of the cycles which have occurred in every adult subject in the Orange Park colony. These changes and fluctuations were found to be associated most definitely and conspicuously with age, with pregnancy and with the season of the year. Counterparts are seen in other primates, including man, but almost without exception the effects which have been described are stated in terms of the entire reproductive cycle. With the chimpanzee it has been possible to go much farther since the sexual cycle in this species is divided into four easily identified parts corresponding to *a*) a short initial phase of follicular inactivity, *b*) a phase of follicular activity, *c*) a luteal phase and *d*) menstruation. By the simple expedient of determining the effect of such factors as age, pregnancy, season, illness and social relations on each of the four phases, in itself a contribution, it is possible to ascertain the extent to which a change or modification in the cycle as a whole is attributable to a change in one or more of its parts.

While these aspects of the subject were being investigated, collateral lines of inquiry added other details to our knowledge of the physiology of reproduction in this species. Data are given which emphasize the importance of differences between individuals for the character of the cycle. The frequency of sterile matings and abortion has been compared with the extent to which each part of the cycle contributed to the length of the entire cycle and the subject is considered from the standpoint of the period of adolescent sterility (Hartman, 1931, 1932; Ashley-Montagu, 1939, a; Smith and Ruben-

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stein, 1940). The results from a few observations on the relationship between ovulation and the beginning of perineal detumescence are summarized, and the difficulty of arriving at a conclusion consistent with the views held by earlier investigators of the problem (Zuckerman, 1930, 1937; Zuckerman and Parkes, 1932; Elder and Yerkes, 1936, b; Elder, 1938, a) is emphasized. Eight additional subjects supplement our information on the time of the menarche (Schultz and Snyder, 1935; Spence and Yerkes, 1937; Grether and Yerkes, 1940).

## MATERIAL AND METHODS

The data were taken from the records of 22 intact female chimpanzees which were displaying or had recently displayed cyclic reproductive activity in the Orange Park colony. They are inclusive of, and supplementary to, data used in the preparation of reports by Yerkes (1935), Elder and Yerkes (1936, a and b), Tomilin (1936), Yerkes and Elder (1936), Spence and Yerkes (1937), Elder (1938, a) and Grether and Yerkes (1940). The only subjects in which cyclic activity was not being shown during April, 1942, the last month for which data were taken, were 6 animals oöphorectomized about a year previously. When the data were being summarized early in 1942 the age of the 22 subjects varied. The 16 for which the date of birth is hypothetical were estimated to be 13 to 29 years old. The 6 animals whose date of birth is known were 9 to 16 years old. The date of the first menstruation is unknown for only 6 subjects. In the remaining 16 it occurred from one-half to 11.5 years previously.

A few data were obtained from subjects other than those referred to above and in one case data were taken after April 30, 1942. Beta, who recently menstruated for the first time, was added to the group for which the time of the menarche is reported. Dwina, a former member of the colony whose history is given by Tinklepaugh (1933), is included among the animals for which the period of adolescent sterility is given. In order to facilitate reference to the sexual status of the different subjects during the cycles immediately prior to laparotomy, the genital record of Lita for the period June 11, 1942 to March 3, 1943 is given in table 3. This part of her record, obtained after the mean values had been determined, was not used in the calculations summarized in tables 2, 5 and 9.

Except for 2 days, Sept. 28 and 29, 1934, examination of the genital condition was made daily beginning May 18, 1931. The procedure was standardized early in the history of the colony and is described by Elder and Yerkes (1936, b). For the purpose of this article it is sufficient to say that the genital condition was recorded as being *a*) quiescent when no perineal turgescence was being shown, *b*) tumescent during the period of development of the perineal or genital swelling, *c*) at a maximum during the period of taut swelling, *d*) detumescent during the period of genital regression, and *e*) menstruating when bleeding per vagina was evident externally.

The decision to consider the cycle as beginning with the first day that bleeding was no longer visible externally is a departure from the custom of regarding the first day of menstruation as the first day of the cycle. This break appears to have been made first by Allen (1927) in his study of menstruation in the Rhesus monkey and later by Zuckerman (1930), but

the precedent has not been followed. To the writers this treatment of the cycle seems justifiable and desirable. In man menstruation has been the only end-point from which the cycle could be established and, despite attempts to ascertain means by which the time of ovulation can be determined (Papanicolaou, 1933; Burr, Musselman, Barton and Kelly, 1937; Rubenstein, 1937; Benedek and Rubenstein, 1942; Langman and Burr, 1942), menstruation is still regarded as the most accurate and generally acceptable point of reference in the human female. Actually, however, its occurrence depends on uterine changes regulated by ovarian activity (Allen, Hisaw and Gardner, 1939) and it is the final rather than the initial event of the cycle. In this study the maintenance of the proper relationship between menstruation and the cycle has had the advantage of facilitating efforts to correlate the various parts of the cycle.

The recurrence of a well defined genital swelling and menstruation permits the division of the cycle into four parts or phases. This gives the chimpanzee a value for such an investigation that is offered by baboons, mangabeys, several members of the genus Macaca (Pocock, 1925; Zuckerman, 1930) and apparently by gorillas (Noback, 1936; Raven, 1936), but not by the Rhesus monkey or man, although Carpenter (1942, a) refers to its occurrence in some Rhesus monkey females during the prime of life. The first part is called the preswelling phase. It is the period of genital quiescence beginning the first day after the external signs of bleeding have disappeared and terminating with the beginning of tumescence. It is assumed, from what has been seen of the ovarian condition during the cycle (unpublished observations), to be either a period of follicular quiescence or a period during which the stimulus from the growing follicle is inadequate to initiate tumescence. The second or swelling phase, that of tumescence and the genital swelling, is terminated by the beginning of detumescence. In most cycles the swelling phase is believed to coincide with the follicular phase so closely that the two terms could be used interchangeably. We shall point out, however, that this is not always true. In instances the follicular phase is terminated by ovulation before the beginning of detumescence, but because identification of such cases cannot be made by any known external signs, whereas the limits of the genital swelling are easily detected, we have chosen to use the beginning of tumescence and the beginning of detumescence as end-points and refer to this part of the cycle as the swelling phase. The third phase is that of detumescence and the period of genital quiescence which precedes menstruation and is called the postswelling phase. Except for cases in which ovulation precedes the beginning of detumescence, or in which anovulatory cycles occur this phase is believed to coincide with the luteal phase, but for the reason that the term follicular phase is avoided, the designation postswelling phase is thought to be preferable to luteal phase. The fourth phase is that of menstruation.

Every cycle taken from the Laboratory records is complete in that the interval which is represented began with the first day after the termination of the bleeding period, included a single period of swelling and ended with the last day of menstruation. When menstruation was not observed, as happened occasionally in several of those bleeding lightly, two cycles were excluded from the data from which the length of the cycle as a whole was calculated. On the other hand, no cycle was disregarded because of a prolonged period of

genital quiescence following menstruation, provided tumescence, detumescence and menstruation subsequently occurred in this order.

During the years covered by this study the subjects displayed 653 cycles for which data as described above are complete. The records of each phase, however, exceed this number (tables 5 and 9). The discrepancy is explained by the circumstance that the swelling phase, the postswelling phase and the menstruation which immediately preceded the first complete cycle after an interruption are included, as are the preswelling and swelling phases of cycles during which a fertile mating took place or during which, for some other reason, menstruation was not observed.

In that part of the investigation dealing with the effect of age, the length of each phase was recorded for each subject according to the 6-month period after the menarche in which the phase began. In the part dealing with the effect of season, the length of each phase was recorded for each subject according to the month in which the phase began. The mean length of the phases for each 6-month period and for each month was then determined for each animal. These means were used in the determination of the means and standard errors for all of the animals during each 6-month period (table 2), for all of the animals during each month (table 5) and for each subject during its entire reproductive life through April 30, 1942 (table 9). The calculations were made to the second decimal place, but in the preparation of the tables, the values were rounded off to the nearest whole number. In every table the means are given in days.

In the study of the effect of age the mean length of the phases during the first 4 years after the menarche was compared with that during the fifth through the eighth year, but when the significance of the differences was tested the 7 subjects were chosen for which there were data in at least four 6-month periods during each of the 4-year periods. In addition, because the effect of pregnancy weighted the data from the latter years, the first cycles after pregnancy were omitted before determining the means used for the calculation of the differences.

In the part of the study dealing with the effect of season, the mean length of the phases for the 'winter' months, October through March, was compared with that for the 'summer' months, April through September. When the differences were tested for significance the data from all of the subjects were included. The "t" tables in Rider (1939) were used for all determinations of significance. They give the probability of a deviation greater than "t" in one direction.

The authors are glad to express their appreciation to Mr. William D. Orbison of the Departments of Psychology, Yale University and Connecicut College for advice with respect to the statistical treatment of the data.

The Laboratory records were the basis for all parts of the investigation, save the correlation of ovarian condition with the genital status as seen externally. For this purpose a series of operations was performed by Drs. Donald M. Baldwin and F. Gordon King of Jacksonville, assisted by Mrs. Baldwin and Mrs. King. To all we are deeply grateful for their skilled and indispensable assistance.

## OBSERVATIONS

*Influence of age.* The changes in the reproductive cycle during the first 8 years after the menarche are shown in tables 1 and 2. The

TABLE 1. REPRODUCTIVE CYCLES OF THE SUBJECT WENDY DURING 8 YEARS AFTER THE MENARCHE
(First menstruation, June 22–25, 1931)

| First Day of Cycle[1] | Preswelling Phase | Swelling Phase | Postswelling Phase | Menstruation | Entire Cycle |
|---|---|---|---|---|---|
| | | 16 | 6 | 4 | |
| 6/26/31 | 11 | 18 | 3 | 3 | 35 |
| 7/31/31 | 18 | 22 | 3 | 6 | 49 |
| 9/18/31 | 10 | 16 | 4 | 6 | 36 |
| 10/24/31 | 9 | 18 | 4 | 4 | 35 |
| 11/28/31 | 9 | 17 | 5 | 5 | 36 |
| 1/ 3/32 | 5 | 21 | 6 | 5 | 37 |
| 2/ 9/32 | 5 | 20 | 6 | 5 | 36 |
| 3/16/32 | 3 | 17 | 10 | 3 | 33 |
| 4/18/32 | 3 | 15 | 9 | 5 | 32 |
| 5/20/32 | 3 | 13 | 11 | 3 | 30 |
| 6/19/32 | 4 | 17 | 10 | 3 | 34 |
| 7/23/32 | 4 | 15 | 11 | 3 | 33 |
| 8/25/32 | 2 | 21 | 9 | 2 | 34 |
| 9/28/32 | 5 | 13 | 12 | 2 | 32 |
| 10/30/32 | 9 | 13 | | | |
| *Pregnancy and lactation occurred* | | | | | |
| | | 25 | 9 | 4 | |
| 9/21/34 | 9 | 15 | 11 | 3 | 38 |
| 10/29/34 | 7 | 15 | 11 | 2 | 35 |
| 12/ 3/34 | 9 | 13 | 12 | 2 | 36 |
| 1/ 8/35 | 5 | 13 | 10 | 3 | 31 |
| 2/ 8/35 | 6 | 13 | 13 | 2 | 34 |
| 3/14/35 | 2 | 14 | 13 | 3 | 32 |
| 4/15/35 | 2 | 15 | 11 | 2 | 30 |
| 5/15/35 | 0 | 14 | 13 | 4 | 31 |
| 6/15/35 | 0 | 14 | | | |
| *Pregnancy and lactation occurred* | | | | | |
| | | 26 | 7 | 2 | |
| 11/27/36 | 9 | 14 | 12 | 2 | 37 |
| 1/ 3/37 | 2 | 15 | 12 | 4 | 33 |
| 2/ 5/37 | 4 | 12 | 12 | 3 | 31 |
| 3/ 8/37 | 3 | 14 | 12 | 4 | 33 |
| 4/10/37 | 0 | 14 | 13 | 3 | 30 |
| 5/10/37 | 0 | 14 | | | |
| *Pregnancy and lactation occurred* | | | | | |
| | | 39 | 12 | 3 | |
| 10/ 5/38 | 1 | 17 | 12 | 2 | 32 |
| 11/ 6/38 | 0 | 16 | 12 | 1 | 29 |
| 12/ 5/38 | 6 | 13 | 11 | 1 | 31 |
| 1/ 5/39 | 3 | 15 | 13 | 3 | 34 |
| 2/ 8/39 | 1 | 14 | 12 | 1 | 28 |
| 3/ 8/39 | 1 | 14 | 13 | 4 | 32 |
| 4/ 9/39 | 1 | 12 | 13 | 1 | 27 |
| 5/ 6/39 | 1 | 15 | 12 | 3 | 31 |
| 6/ 6/39 | 0 | 13 | 13 | 2 | 28 |

[1] This is the date on which external vaginal bleeding was no longer visible.

former is the protocol of a single subject, Wendy, whose history is as representative as any in the colony of a normal healthy animal at the height of reproductive life. Table 2 is a summary prepared from the records of the 16 animals for whom the date of the first menstruation is known. A cursory examination of either table reveals the nature of the changes. The preswelling and swelling phases and menstruation became significantly shorter, particularly during the first year. The

postswelling phase became longer, but the change in the latter was not sufficient to offset that in the other three phases, with the result that the cycle became shorter.

Although differences between individuals were numerous, there is no animal in which the trends described above are not clear in 1 or more of the 4 phases; not even irregularities attributable to season have obscured the picture. The most conspicuous difference is in the rate of change from the condition in the adolescent animal to that in most older subjects. This is illustrated by a comparison of the records

TABLE 2. LENGTH OF PHASES OF THE REPRODUCTIVE CYCLES IN 6-MONTH INTERVALS AS DISPLAYED BY 16 CHIMPANZEES DURING THE FIRST 8 YEARS AFTER THE MENARCHE

| 6-Month Periods After the Menarche | Preswelling Phase | | Swelling Phase | | Postswelling Phase | | Menstruation | | Entire Cycle | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Mean | No. | Mean | No. | Mean | No. | Mean | No. | Mean |
| 1 | 61 | 20 ± 3.86 | 72 | 26 ± 1.27 | 62 | 6 ± 0.18 | 62 | 4 ± 0.16 | 58 | 50 ± 4.38 |
| 2 | 52 | 8 ± 0.49 | 59 | 20 ± 0.71 | 53 | 7 ± 0.29 | 53 | 4 ± 0.20 | 48 | 40 ± 1.10 |
| 3 | 41 | 7 ± 0.39 | 43 | 18 ± 0.51 | 44 | 9 ± 0.21 | 41 | 4 ± 0.24 | 35 | 38 ± 0.88 |
| 4 | 30 | 5 ± 0.35 | 34 | 20 ± 0.61 | 28 | 9 ± 0.47 | 30 | 4 ± 0.22 | 25 | 37 ± 0.68 |
| 5 | 27 | 7 ± 0.47 | 27 | 20 ± 0.53 | 28 | 10 ± 0.12 | 27 | 3 ± 0.21 | 26 | 38 ± 0.40 |
| 6 | 26 | 13 ± 4.35 | 32 | 21 ± 1.07 | 26 | 11 ± 0.33 | 27 | 3 ± 0.29 | 21 | 40 ± 1.43 |
| 7 | 32 | 6 ± 0.93 | 35 | 17 ± 0.48 | 30 | 12 ± 0.26 | 31 | 3 ± 0.20 | 28 | 37 ± 1.04 |
| 8 | 27 | 5 ± 0.51 | 31 | 17 ± 0.46 | 28 | 11 ± 0.30 | 27 | 3 ± 0.20 | 24 | 37 ± 0.70 |
| 9 | 20 | 6 ± 0.61 | 21 | 18 ± 0.43 | 18 | 11 ± 0.28 | 19 | 2 ± 0.29 | 15 | 37 ± 0.43 |
| 10 | 20 | 4 ± 0.44 | 22 | 20 ± 1.12 | 19 | 11 ± 0.36 | 20 | 3 ± 0.19 | 15 | 36 ± 0.94 |
| 11 | 21 | 14 ± 3.62 | 25 | 19 ± 0.61 | 22 | 11 ± 0.32 | 21 | 3 ± 0.15 | 22 | 46 ± 4.12 |
| 12 | 30 | 5 ± 0.62 | 30 | 19 ± 1.19 | 29 | 11 ± 0.21 | 30 | 3 ± 0.20 | 25 | 38 ± 1.46 |
| 13 | 20 | 4 ± 0.49 | 25 | 19 ± 0.74 | 21 | 12 ± 0.20 | 20 | 3 ± 0.30 | 20 | 37 ± 0.48 |
| 14 | 18 | 4 ± 0.33 | 20 | 18 ± 0.68 | 18 | 12 ± 0.41 | 18 | 3 ± 0.22 | 17 | 35 ± 0.79 |
| 15 | 26 | 4 ± 0.58 | 28 | 18 ± 0.45 | 25 | 11 ± 0.36 | 27 | 3 ± 0.26 | 23 | 34 ± 0.61 |
| 16 | 23 | 3 ± 0.28 | 25 | 18 ± 0.55 | 25 | 12 ± 0.19 | 22 | 3 ± 0.31 | 17 | 35 ± 0.81 |
| *Significance of the differences between the first 4 years and the 5th to 8th years* | | | | | | | | | | |
| | DM = 5.9 Significant $t$ = 2.50 with 6 d.f. 2.5% level = 2.45 | | DM = 2.6 Significant $t$ = 2.14 with 6 d.f. 2.5% level = 2.45; 5.0% level = 1.94 | | DM = −2.4 Significant $t$ = −4.66 with 6 d.f. 0.5% level = 2.85 | | DM = 0.9 Significant $t$ = 4.35 with 6 d.f. 0.5% level = 2.85 | | DM = 7.4 Significant $t$ = 2.26 with 6 d.f. 2.5% level = 2.45; 5.0% level = 1.94 | |

of two members of the colony. In Wendy (table 1) about 9 months elapsed after the first menstruation before the postswelling phases became as long as 10 days and by this time the preswelling and swelling phases were noticeably shorter. In Lita (table 3) the postswelling phase became longer, but not once during the first 2.66 years did it attain a length of 10 days unless the postswelling phase during the cycle in which conception occurred would have been of this length. The change in the preswelling phase was also slower than in Wendy.

The rate of maturation was slow in at least two other subjects, Beta and Gamma. The fact is mentioned because we cannot exclude the possibility that Lita's slow rate of maturation was due to secondary factors. Five years earlier, when she was nearly 7 years old, she was

given 37,800 I.U. of estrone over a 3-month period (Elder, 1938, b). Regression of the artificially produced genital swelling was complete within 7 days after the last injection and, aside from the prolonged period of adolescence, her subsequent history was uneventful. We do not know whether this abnormality was a sequela of the hormonal treatment. Indirect evidence suggests that the likelihood is remote. In the rat, permanent effects follow the administration of estrogen

TABLE 3. REPRODUCTIVE CYCLES OF THE SUBJECT LITA DURING 2.66 YEARS AFTER THE MENARCHE

(First menstruation, April 4–10, 1940)

| First Day of Cycle[1] | Preswelling Phase | Swelling Phase | Postswelling Phase | Menstruation | Entire Cycle |
|---|---|---|---|---|---|
| | | 37 | 2 | 7 | |
| 4/11/40 | 2 | 26 | 2 | 4 | 34 |
| 5/15/40 | 7 | 23 | 4 | 4 | 38 |
| 6/22/40 | 13 | 22 | 3 | 4 | 42 |
| 8/ 3/40 | 11 | 21 | 3 | 4 | 39 |
| 9/11/40 | 7 | 26 | 3 | 5 | 41 |
| 10/22/40 | 19 | 27 | 3 | 4 | 53 |
| 12/15/40 | 11 | 23 | 2 | 3 | 39 |
| 1/23/41 | 21 | 23 | 5 | 3 | 52 |
| 3/16/41 | 10 | 19 | 6 | 3 | 38 |
| 4/23/41 | 6 | 22 | 6 | 4 | 38 |
| 5/31/41 | 10 | 18 | 7 | 3 | 38 |
| 7/ 8/41 | 10 | 16 | 7 | 3 | 36 |
| 8/13/41 | 4 | 17 | 5 | 2 | 28 |
| 9/10/41 | 6 | 17 | 7 | 4 | 34 |
| 10/14/41 | 4 | 19 | 7 | 3 | 33 |
| 11/16/41 | 4 | 19 | 6 | 4 | 33 |
| 12/19/41 | 9 | 16 | 7 | 4 | 36 |
| 1/24/42 | 6 | 21 | 7 | 4 | 38 |
| 3/ 3/42 | 4 | 22 | | | |
| *Pregnancy; abortion on 29th day after conception* | | | | | |
| | | 17 | 5 | 2 | |
| 6/11/42 | 11 | 18 | 5 | 2 | 36 |
| 7/17/42 | 6 | 23 | 5 | 2 | 36 |
| 8/22/42 | 13 | 20 | 6 | 3 | 42 |
| 10/ 3/42 | 7 | 27 | 8 | 2 | 44 |
| 11/16/42 | 12 | 17 | 7 | 4 | 40 |
| 12/26/42 | 15 | 19 | 7 | 2 | 43 |
| 2/ 7/43 | 9 | 16+ | | | |

[1] Dated from cessation of external vaginal bleeding.

only when the hormone is given in adequate quantities beginning within the first 10 days after birth. The effect of similar injections into older animals is only transitory (Wilson, 1943). In mature human females large quantities of estrogen do not appear to have caused any permanent change (Zondek, 1935; Gillman, 1942), a temporary amenorrhea was produced, but after a short time the cycles were again regular.

When the short postswelling phases during the period immediately after the menarche were first encountered it was assumed, from the frequency of anovulatory cycles during adolescence in the Rhesus monkey (Allen, 1927; Hartman, 1928, 1931, 1932; Smith and Ruben-

stein, 1940) and from the evidence reviewed by Hartman (1931) and Ashley-Montagu (1939, a) for the human female, that the short postswelling phases during this period in the chimpanzee are also attributable to anovulatory cycles. It soon became apparent, however, that this assumption was not warranted, especially when the length of the postswelling phase was 6 days or more. In numerous instances fertile matings occurred during the period when these short postswelling phases were being shown. Additional evidence for the occurrence of ovulation was obtained by the direct observation of the ovaries in the subject Lita following rupture of the follicle in the cycle which began Feb. 7, 1943 (table 3). One newly-formed corpus luteum and 4 corpora albicantia or corpora fibrosa were seen. If the latter may be assumed to have been associated with the last 4 complete cycles, they were formed as corpora lutea during cycles when the length of the postswelling phase varied from 6 to 8 days.

Cycles in which the postswelling phase was shorter than 6 days may have been anovulatory. No direct observations have been possible, but in 5 cases in which estrogen withdrawal was accomplished by removal of the ovaries between the fifth and fourteenth day of the swelling phase, the length of the postswelling phase was 6 days or less. It was 1 day in an animal from which the single remaining ovary was removed the eighth day, 3 days in 3 animals from which the ovaries were removed the fifth (1 subject) and fourteenth days (2 subjects), and 6 days in an animal from which the ovaries were removed the twelfth day. We cannot account for the lack of relationship between the time of the operation with respect to the swelling phase and the length of the postswelling phase following the operation.

TABLE 4. LENGTH OF PHASES IN THE LAST REPRODUCTIVE CYCLES BEFORE PREGNANCY AND THE FIRST AFTER PREGNANCY

| | Preswelling Phase | | Swelling Phase | | Postswelling Phase | | Menstruation | |
|---|---|---|---|---|---|---|---|---|
| | No. | Mean | No. | Mean | No. | Mean | No. | Mean |
| Before pregnancy | 48 | 4 ± 0.43 | 48 | 17 ± 0.53 | 47 | 11 ± 0.35 | 47 | 3 ± 0.17 |
| After pregnancy | 42 | 11 ± 2.25 | 40 | 23 ± 1.21 | 40 | 9 ± 0.45 | 42 | 3 ± 0.14 |

*Effect of pregnancy.* The change in the length of the various phases of the cycle that frequently followed pregnancy, or pregnancy and lactation, is summarized in table 4 and is shown for a single subject in table 1. Following 35 of 48 pregnancies there was a temporary reversion in at least one of the phases, except menstruation, to the condition seen during the first months after the menarche; the preswelling and swelling phases were long and the postswelling phases were short. When such a change was shown the return to the normal condition usually occurred by the time of the second cycle. In only 5 of the 35 cases did recovery occupy the time of more than one cycle. The effect of pregnancy was more pronounced in young than in older subjects.

*Influence of season.* The influence of season is shown by the data summarized in table 5. The principal difference between the influence of season and that of age or pregnancy is that only the preswelling phase was affected by the season. However the fluctuations in the length of the preswelling phase were so great that the difference in the length of the cycle during the summer and winter months is also significant. As in the case of the other factors, not all animals reacted

TABLE 5. SEASONAL VARIATION IN THE LENGTH OF PHASES IN THE REPRODUCTIVE CYCLES OF 22 SUBJECTS

| Month | Preswelling Phase | | Swelling Phase | | Postswelling Phase | | Menstruation | | Entire Cycle | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Mean | No. | Mean | No. | Mean | No. | Mean | No. | Mean |
| January | 57 | 11±2.12 | 67 | 17±0.34 | 63 | 9±0.27 | 56 | 3±0.13 | 52 | 38±0.73 |
| February | 61 | 6±0.38 | 67 | 19±0.48 | 55 | 10±0.30 | 60 | 3±0.12 | 56 | 38±0.57 |
| March | 62 | 5±0.67 | 82 | 19±0.33 | 68 | 10±0.30 | 60 | 3±0.12 | 55 | 39±1.69 |
| April | 61 | 5±0.30 | 72 | 18±0.33 | 65 | 10±0.25 | 71 | 3±0.11 | 49 | 35±0.44 |
| May | 66 | 4±0.32 | 67 | 18±0.39 | 60 | 10±0.25 | 61 | 3±0.14 | 53 | 35±0.52 |
| June | 63 | 5±0.31 | 70 | 17±0.32 | 61 | 10±0.30 | 63 | 3±0.16 | 52 | 36±0.60 |
| July | 62 | 4±0.26 | 73 | 18±0.37 | 61 | 10±0.31 | 64 | 3±0.15 | 47 | 35±0.45 |
| August | 63 | 6±0.43 | 81 | 18±0.42 | 65 | 10±0.28 | 60 | 3±0.14 | 56 | 37±0.54 |
| September | 70 | 6±0.70 | 81 | 17±0.25 | 70 | 10±0.23 | 73 | 3±0.15 | 60 | 35±0.36 |
| October | 79 | 6±0.32 | 69 | 17±0.34 | 71 | 10±0.21 | 73 | 3±0.13 | 67 | 36±0.51 |
| November | 61 | 9±0.94 | 69 | 18±0.30 | 65 | 9±0.19 | 67 | 3±0.16 | 53 | 42±1.59 |
| December | 57 | 14±2.04 | 68 | 20±1.19 | 58 | 9±0.27 | 58 | 3±0.10 | 53 | 43±2.09 |

*Significance of the differences between the periods, October–March and April–September*

| | | | | |
|---|---|---|---|---|
| DM=3.6<br>Significant<br><br>$t$=3.65 with 20 d.f.<br>0.5% level<br>=2.85 | DM=0.6<br>Not significant<br>$t$=1.14 with 20 d.f.<br>10% level<br>=1.33 | DM=−0.3<br>Not significant<br>$t$=−1.60 with 20 d.f.<br>5% level<br>=1.73 | DM=0.2<br>Not significant<br>$t$=1.71 with 20 d.f.<br>5% level<br>=1.73 | DM=4.1<br>Significant<br>$t$=3.45 with 20 d.f.<br>0.5% level<br>=2.85 |

similarly to seasonal change. Wendy and Lita were affected only slightly (tables 1 and 3). In general the effect was greatest during the first years after the menarche, but in instances a recent pregnancy or illness may have made it more pronounced.

A suggestion of the effect of season on the time of the menarche appears from the data in table 6 although the time of the first conception does not seem to have been influenced by the seasonal factor. The number of cases is small, but the frequency with which the menarche occurred during the summer months is contrasted with the lack of correlation between the first conception and season and is believed to be significant. The data in table 5 provide an explanation. Menstruation is dependent on a certain amount of development of the graafian follicle. The fact that the preswelling phase was generally shorter in the summer months suggests that these months are more favorable for the initiation of the follicular growth which is a prerequisite for the first menstruation. Conception, on the other hand, is dependent on ovulation and the presence of a corpus luteum, but as

noted above, there is no evidence that the part of the cycle associated with luteal function is affected by season. Consequently, the lack of relationship between season and the time of the first conception need not be surprising.

Identification of the seasonal factor or factors which are responsible for fluctuations in the preswelling phase would be of interest, especially in view of the influence of length of day and temperature on the reproductive activity of lower mammals (Bissonnette, 1936, 1941; Marshall, 1936, 1937; Moore, Simmons, Wells, Zalesky and Nelson, 1934; Dempsey and Searles, 1943). As far as the chimpanzee is concerned, the establishment of conditions whereby the rôle of these

TABLE 6. THE MONTH OF THE FIRST MENSTRUATION AND OF THE FIRST CONCEPTION FOR 18 AND 13 SUBJECTS, RESPECTIVELY

| Month | First Menstruation | First Conception | Month | First Menstruation | First Conception |
|---|---|---|---|---|---|
| January | 0 | 1 | July | 4 | 0 |
| February | 1 | 1 | August | 3 | 1 |
| March | 1 | 1 | September | 2 | 1 |
| April | 1 | 0 | October | 1 | 1 |
| May | 0 | 2 | November | 1 | 3 |
| June | 3 | 1 | December | 1 | 1 |

factors could be tested separately is not practicable. The only approach which has been possible is a comparison of the records from the Jacksonville Weather Bureau with those of the animals during the years covered by the observations (fig. 1). In this figure curves are given for the mean temperature each month of the year for the 12-year period, 1930–1941, for the mean daily sunlight each month of the year for the 9-year period, 1933–1941, and for the length of day the 15th of each month throughout the year. The fourth curve represents the total number of days the preswelling phase was shown each month of the year for the 11-year period, 1931–1942. This manner of presenting the data supplements that in table 5 in which the mean length of the phases is given according to the month of the year in which the phase began. Both methods reveal the effect of season, but neither demonstrates a relationship to any one of the three seasonal factors. The change could be a consequence of one or of all.

*Illness.* There is abundant evidence from the human female (Novak, 1921; Fluhmann, 1939) that other illnesses as well as glandular disturbances have a marked effect on the reproductive cycle. It does not follow, however, that every illness exerts such an action. If in a colony of animals the illnesses are not in the nature of wasting diseases or intoxications or are not caused by malnutrition, the effects might be slight and not at all indicative of the consequences of other and more serious illnesses. Some such explanation may account for the circumstance that only a few irregularities can unequivocally be attributed to illness.

With the exception of one case diagnosed as hemichorea, the recorded illnesses have been gastrointestinal disturbances associated with an infestation of parasites such as pinworm and Balantidium coli, and colds. The nature of the illness and the length of the phases of the cycles during which the illness was reported is given in table 7. The phases during which the animals were ill are italicized. It will be ap-

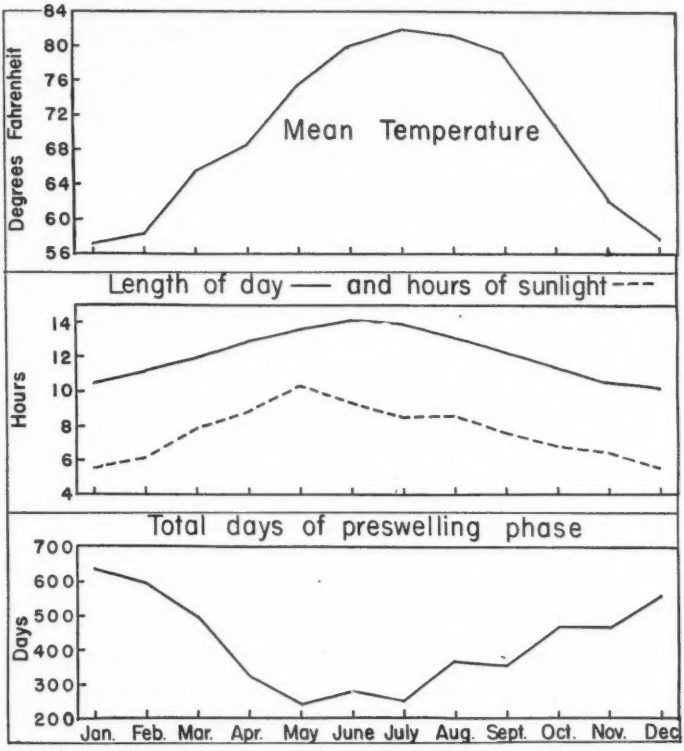


FIG. 1. The mean temperature, the mean daily hours of sunlight, the length of day (sunrise to sunset) the 15th of each month, and the total days per month the preswelling phase was shown May 18, 1931 through March 31, 1942.

parent immediately that the swelling and postswelling phases and menstruation were affected little if at all. The failure of menstruation in Kambi and the long swelling phase and short postswelling phase in Mona are the only deviations which exceed the normal range of variation. In contrast with the general lack of effect of illness on the last three phases of the cycle, is the correlation between illness and the length of the preswelling phase. In 9 of 17 instances in which the subjects were ill during the preswelling phase the length of this phase exceeded the probable error. We cannot be certain, however, that the

great length of this phase in these cases was a consequence of illness because in 7 of the 9 the illness occurred during the winter season. It is a question whether the illness itself prolonged the length of the preswelling phase or whether the animals were not more susceptible to illness during the winter season when the preswelling phase tended to be prolonged. In many instances during these months long preswelling

TABLE 7. LENGTH OF REPRODUCTIVE CYCLE DURING PERIODS OF ILLNESS. THE PHASES DURING WHICH ILLNESS OCCURRED ARE IN ITALICS.

| Subject | Beginning of Illness | Duration in Days | Pre-swelling Phase | Swelling Phase | Post-swelling Phase | Menstruation |
|---|---|---|---|---|---|---|
| | | | *Gastrointestinal* | | | |
| Bentia | 8/ 4/35 | 18 | *15* | 36 | 9 | 2 |
| Cuba | 8/25/33 | 25 | 7 | *16* | *7* | *5* |
| | 3/13/40 | 19 | 4 | *14* | 9 | 2 |
| Dita | 12/ 1/41 | 91 | *132* | 15 | 8 | 2 |
| Fifi | 1/10/35 | 24 | 1 | *19* | *10* | *3* |
| | 11/ 1/40 | 11 | 0 | *11* | *14* | 4 |
| Kambi | 10/13/38 | 71 | *5* | *23* | *?* | *0* |
| | | | *+50* | 24 | 6 | 4 |
| Lia | 10/15/35 | 22 | *6* | *10* | *15* | 1 |
| Mamo | 6/ 6/40 | 5 | *1* | 15 | 12 | 2 |
| Mona | 4/ 3/42 | 29 | 7 | *27* | *3* | *2* |
| | | | *11* | 30 | 10 | 1 |
| Nana | 10/25/40 | 17 | 2 | 15 | *12* | *3* |
| | | | *2* | 16 | 11 | 5 |
| | 2/ 2/42 | 17 | *32* | 17 | 12 | 1 |
| | 4/19/42 | 37 | 2 | *16* | *11* | *5* |
| | | | *3* | *17* | 11 | 6 |
| Nira | 11/18/34 | 19 | *23* | 19 | 7 | 4 |
| | 12/16/35 | 48 | 12 | 15 | *10* | *1* |
| | | | *25* | *19* | 8 | 5 |
| Pati | 12/ 3/40 | 29 | *100* | 18 | 9 | 2 |
| | 10/ 1/42 | 60 | 7 | 13 | 7 | *3* |
| | | | *123* | 12 | 10 | 2 |
| | | | *Respiratory* | | | |
| Fifi | 4/29/40 | 7 | 3 | *16* | ? | ? |
| Pati | 7/20/40 | 6 | 4 | *16* | conception | |
| | | | *Hemichorea* | | | |
| Mimi | 2/ 4/37 | 178 | *10* | *20* | *11* | *5* |
| | | | *5* | *19* | *11* | *2* |
| | | | *9* | *23* | 11 | 4 |

phases were shown when the health of the animals appeared to be excellent.

*Social relations.* The opinion that the character of the primate cycle is affected by social relations (Elder and Yerkes, 1936, b; Yerkes and Elder, 1936; Carpenter, 1942, b) and emotional factors (Gillman, 1935, 1937), as it is in the human female (Latz and Reiner, 1935; Fluhmann, 1939), led to a reëxamination of the data by the procedure employed when the influence of age, pregnancy, season and illness were being studied. The length of phases was compared when the

females were alone, when they were confined with males and when they were caged with other females (table 8). It is clear by inspection that under none of the three conditions of confinement was the mean length of any phase significantly different from that under either of the other conditions of confinement or from the mean for the colony as a whole. We conclude, therefore, that no variation which has thus far been made in social relations or in the frequency of mating has influenced the character of the cycle. During the excitement caused by shifting animals the senior author has seen what he has judged to be an increase in size of the genital swelling similar to that

TABLE 8. MEAN LENGTH OF THE PARTS OF THE CYCLE WHEN THE FEMALES WERE ALONE IN A CAGE, CONFINED WITH A MALE, OR WITH OTHER FEMALES

| | Preswelling Phase | Swelling Phase | Postswelling Phase |
|---|---|---|---|
| Number of phases alone | 66 | 52 | 43 |
| Mean | 7.52 days | 17.19 days | 9.37 days |
| Range | 0–101 days | 10–36 days | 2–15 days |
| Number of phases with a male | 90 | 152 | 84 |
| Mean | 7.51 days | 17.52 days | 8.77 days |
| Range | 0–115 days | 10–36 days | 2–19 days |
| Number of phases with females | 417 | 335 | 301 |
| Mean | 5.75 days | 17.40 days | 10.56 days |
| Range | 0–100 days | 8–61 days | 2–21 days |
| Number of phases under all conditions of confinement | 762 | 866 | 762 |
| Mean | 6.84 days | 18.14 days | 9.69 days |
| Range | 0–137 days | 8–95 days | 1–27 days |

described by Yerkes and Elder, but it is his opinion that such a change is never shown unless tumescence has already begun or is imminent. A detumescence brought about by fighting and leading to an early appearance of complete genital quiescence such as Gillman (1937) describes as occurring in the baboon has not been seen.

*Differences between individuals.* Among the factors suspected of affecting the character of the cycle are those tentatively classified as differences between individuals. In the absence of information that would enable us to be more specific, these differences are ascribed to variations in the endocrine balance and to other chronic peculiarities of function. In the data summarized in this article differences between individuals are seen in the length of the cycle, in the length of the separate phases, in the extent to which the separate phases contribute to the total length of the cycle and in the rate at which maturation occurs. Evidence for differences in the rate of maturation has been cited. Other examples of differences between individuals are taken from the protocols in the files of the Laboratory at Orange Park and from the data in tables 9 and 10. Menstruation was consistently longer in Mimi than in Lia, Josie, Nana and Wendy whose ages are

TABLE 9. LENGTH OF PHASES IN 22 SUBJECTS ARRANGED IN THE ORDER OF AGE AS GIVEN IN TABLE 10

| Subject | Preswelling Phase | | Swelling Phase | | Postswelling Phase | | Menstruation | | Entire Cycle | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Mean | No. | Mean | No. | Mean | No. | Mean | No. | Mean |
| Mona | 35 | 5±0.24 | 41 | 19±0.33 | 35 | 9±0.21 | 35 | 3±0.05 | 28 | 35±0.32 |
| Fifi | 45 | 5±0.90 | 52 | 15±0.12 | 44 | 12±0.26 | 44 | 3±0.10 | 37 | 35±0.84 |
| Dita | 32 | 12±2.53 | 40 | 19±0.35 | 32 | 9±0.12 | 33 | 2±0.08 | 26 | 42±2.92 |
| Pati | 45 | 12±1.82 | 52 | 13±0.13 | 44 | 10±0.26 | 44 | 3±0.08 | 37 | 33±2.04 |
| Nana | 55 | 4±0.32 | 60 | 16±0.11 | 53 | 9±0.12 | 54 | 3±0.08 | 45 | 33±0.48 |
| Josie | 37 | 7±0.55 | 48 | 16±0.29 | 39 | 12±0.24 | 39 | 2±0.10 | 28 | 36±0.33 |
| Mimi | 58 | 9±1.12 | 59 | 22±0.39 | 57 | 9±0.10 | 57 | 6±0.09 | 54 | 46±1.43 |
| Wendy | 45 | 4±0.30 | 52 | 16±0.30 | 46 | 10±0.18 | 46 | 3±0.08 | 39 | 33±0.28 |
| Lia | 48 | 9±1.33 | 55 | 16±0.38 | 48 | 14±0.24 | 48 | 2±0.07 | 41 | 39±1.11 |
| May | 54 | 4±0.17 | 61 | 22±0.49 | 54 | 11±0.12 | 54 | 3±0.06 | 47 | 39±0.60 |
| Cuba | 60 | 5±0.20 | 67 | 17±0.11 | 58 | 9±0.17 | 60 | 3±0.04 | 51 | 34±0.23 |
| Nira | 70 | 6±0.36 | 70 | 17±0.09 | 70 | 11±0.08 | 70 | 4±0.04 | 69 | 37±0.33 |
| Bentia | 30 | 6±0.45 | 37 | 22±0.69 | 29 | 12±0.28 | 30 | 2±0.12 | 22 | 41±1.09 |
| Soda | 20 | 10±1.48 | 24 | 23±2.28 | 20 | 9±0.41 | 20 | 3±0.17 | 16 | 45±4.09 |
| Mamo | 30 | 7±1.68 | 36 | 18±0.27 | 29 | 11±0.24 | 29 | 2±0.11 | 22 | 35±0.65 |
| Kambi | 23 | 12±4.08 | 24 | 20±0.45 | 23 | 8±0.19 | 23 | 4±0.16 | 21 | 38±1.02 |
| Bimba | 12 | 7±0.73 | 15 | 22±0.87 | 12 | 9±0.36 | 12 | 4±0.35 | 9 | 41±1.53 |
| Lita | 19 | 9±0.70 | 21 | 21±0.68 | 19 | 5±0.28 | 19 | 4±0.14 | 18 | 38±0.84 |
| Bula | 15 | 5±0.36 | 16 | 17±0.49 | 15 | 8±0.39 | 15 | 3±0.42 | 14 | 33±0.99 |
| Alpha | 11 | 4±0.55 | 20 | 16±0.34 | 17 | 10±0.50 | 17 | 4±0.27 | 14 | 33±0.62 |
| Gamma | 3 | 6±1.01 | 4 | 17±0.74 | 4 | 7±1.02 | 4 | 2±0.25 | 3 | 31±0.43 |
| Helene | 12 | 5±0.88 | 13 | 16±0.59 | 13 | 9±0.55 | 13 | 4±0.35 | 12 | 35±0.79 |
| Total | 762 | 7±0.08 | 866 | 18±0.08 | 762 | 10±0.06 | 766 | 3±0.03 | 653 | 37±0.14 |

about the same. The preswelling and swelling phases were generally longer in Bentia and Soda than in Cuba and May who are of comparable age. The combined length of the preswelling and swelling phases in Pati was greater than in most other animals, but this difference is accounted for by the unusual length of the preswelling phase. Once tumescence began it progressed rapidly and the swelling phase was generally shorter than that in any other subject. The mean length of the postswelling phase was greater in Lia than in any other member of the colony, even though 8 animals are older and therefore might be expected to have displayed longer rather than shorter postswelling phases. The differences in the extent to which the separate phases contribute to the length of the cycle can also be seen from the data summarized in table 10. Not only is the variation great in the colony as a whole, but the subjects are listed according to age and even within relatively small groups of about the same age the differences are great.

*Length and regularity of the reproductive cycle.* The mean length of the 653 cycles is 37.28±0.14 days, range 22 to 187 days. The median is 35 days and the peak of the curve which is skewed to the right, largely because of the long cycles frequently seen in young animals during the winter season, is at 33 days. The influence of age, pregnancy and season is so great and the importance of differences between individuals is so obvious that any mean such as 37.28 days for all the cycles

TABLE 10. THE EXTENT TO WHICH THE SEPARATE PHASES CONTRIBUTED TO THE TOTAL LENGTH OF THE CYCLE IN 22 SUBJECTS ARRANGED ACCORDING TO AGE

| Subject | Age in Years as of April 30, 1942 | Pre-swelling Phase | Swelling Phase | Post-swelling Phase | Menstruation |
|---|---|---|---|---|---|
| | | % | % | % | % |
| Mona | 29[1] | 13 | 53 | 25 | 7 |
| Fifi | 24[1] | 15 | 43 | 34 | 8 |
| Dita | 22[1] | 34 | 45 | 21 | 5 |
| Pati | 22[1] | 36 | 40 | 31 | 8 |
| Nana | 21[1] | 12 | 50 | 27 | 9 |
| Josie | 20[1] | 19 | 45 | 32 | 5 |
| Mimi | 19[1] | 20 | 48 | 20 | 12 |
| Wendy | 19[1] | 13 | 47 | 31 | 8 |
| Lia | 18[1] | 22 | 41 | 35 | 5 |
| May | 17[1] | 9 | 56 | 29 | 8 |
| Cuba | 16 | 14 | 49 | 28 | 9 |
| Nira | 16[1] | 16 | 45 | 29 | 11 |
| Bentia | 16[1] | 15 | 53 | 28 | 4 |
| Soda | 15[1] | 21 | 52 | 20 | 7 |
| Mamo | 14[1] | 19 | 50 | 31 | 7 |
| Kambi | 13[1] | 31 | 54 | 20 | 12 |
| Bimba | 13[1] | 6 | 53 | 21 | 10 |
| Lita | 12 | 23 | 56 | 13 | 10 |
| Bula | 12 | 15 | 52 | 23 | 9 |
| Alpha | 11 | 11 | 48 | 31 | 11 |
| Gamma | 9 | 19 | 54 | 22 | 5 |
| Helene | 9 | 14 | 47 | 25 | 12 |
| Total | | 18 | 49 | 26 | 8 |

[1] Estimated.

requires qualification. It does provide a basis for a comparison of the cycle in the chimpanzee with that in other primates when the sample is equally heterogeneous. In addition, there is no reason for doubting but that the figure is close to the mean that would be found in any comparable group of chimpanzees. On the other hand, when homogeneity is attained with respect to the factors which influence the length of the cycle, a different value is obtained. The mean for a group of adolescent animals is higher, that for a group of older subjects is lower (table 2). It is probable that as other dichotomies are established, further refinement will be possible.

Utilizing existing data, (Hartman, 1932; Knaus, 1934; Arey, 1939; Fluhmann, 1939) an effort was made to account for the difference between the cycle length of the chimpanzee as compared with the Rhesus monkey and human female. Menstruation averages at least a day less in the chimpanzee, and, if the numerous estimates of the time of ovulation are correct, the interval between ovulation and menstruation is also less in the chimpanzee, 11 as compared with 14 days in the monkey and the human female. The longer cycle in the chimpanzee, therefore, is accounted for by a difference in the preswelling (follicular quiescence) and swelling (follicular growth) phases whose combined length, estimated to average about 25 days, is more than twice the 9 to 10-day follicular phase in the other two species. It will be clear

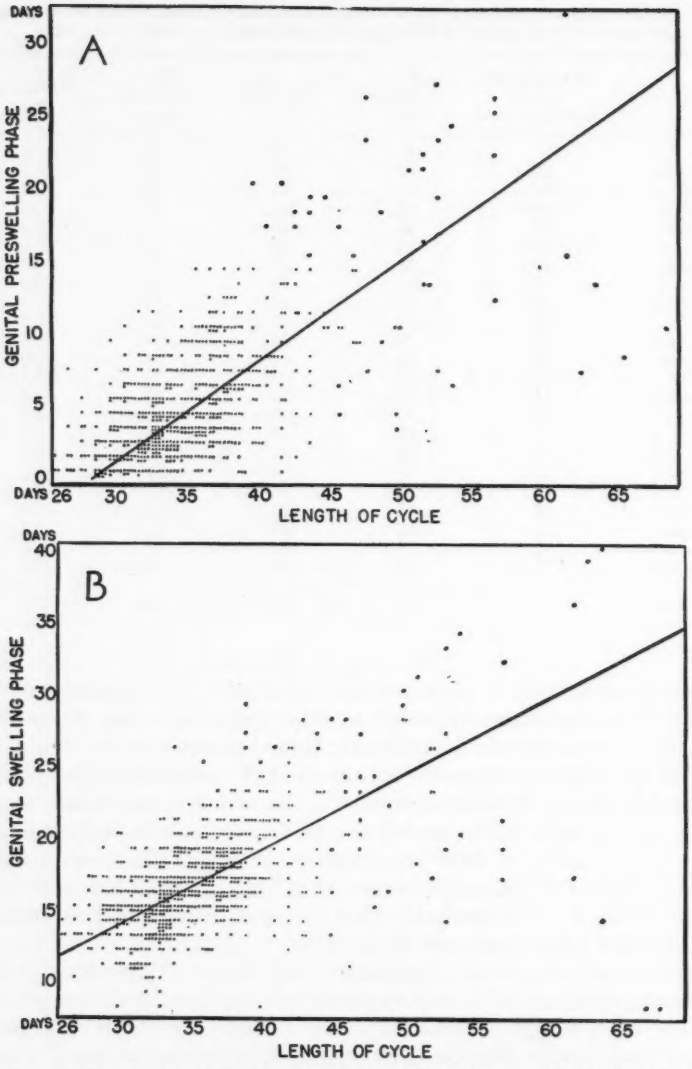


FIG. 2. Correlation of length of genital swelling phases with the length of the cycle. A. Preswelling phase. B. Swelling phase.

from what has been said previously that the relationship varies with age, but even in a group of fully mature chimpanzees in which the post-swelling phase averages nearly 12 days, the length of the combined preswelling and swelling phases averages 22 days.

The frequency of irregularities has been commented on by almost every investigator of cycle length in the primates (Hartman, 1932;

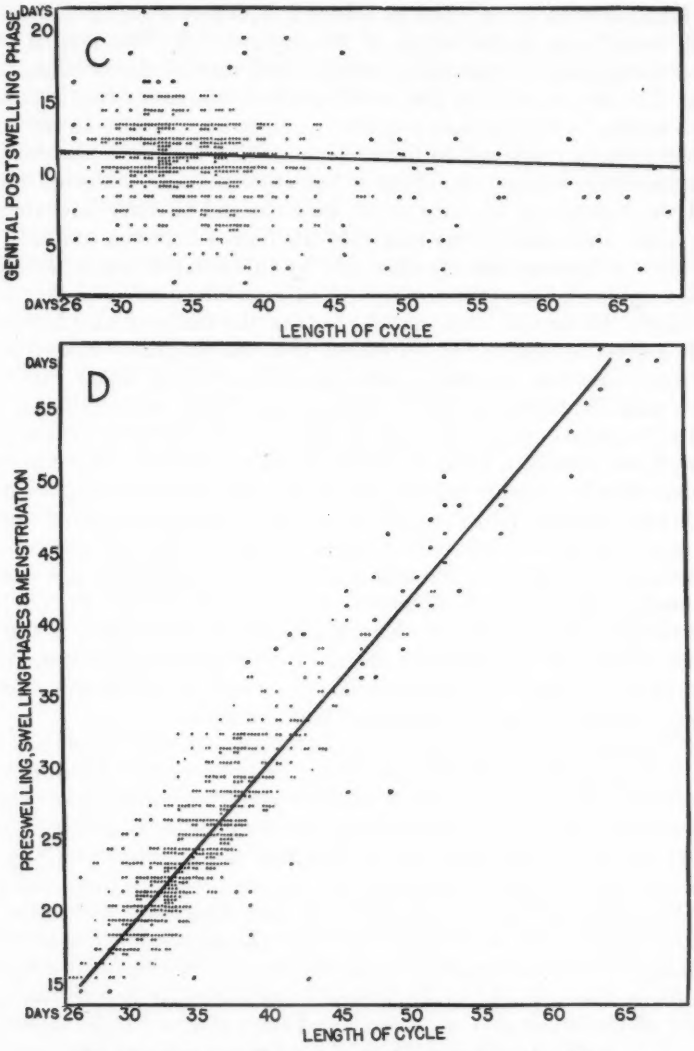


FIG. 2. C. Postswelling phase. D. Preswelling and swelling phases and menstruation.

Gillman, 1935, 1937; Fluhmann, 1934, 1939; King, 1926, 1933; Arey, 1939). The chimpanzee presents no exception, but in view of the number and diversity of the factors influencing the cycle, and the fact that each of the separate phases can and frequently does vary independently of the others, a regularity of length much greater than that shown in tables 1 and 3, or by the subject Nira, would be surprising.

Examination of the data in tables 2, 4, 5 and 9 and in figure 2 reveals something of the origin of this irregularity. The length of the preswelling phase is the most variable and that of the swelling phase next. The duration of the postswelling phase and menstruation are the most stable. It follows that most of the large fluctuations in the length of the cycle are related to fluctuations in the first two phases. Once detumescence begins, the cycle is less influenced by external factors and the balance of its course can be expected to vary little beyond the limits imposed by age and the differences between animals.

More or less completely obscured by this irregularity in the length of the cycle is a certain trend toward constancy of character in individuals. Hints that this may be true in the monkey and human female are contained in the statement that the length of menstruation is fairly constant in many individuals (Hartman, 1932; McCance, Love and Widdowson, 1937; Fluhmann, 1939), although Hartman and Fluhmann express the opinion that a grouping of individuals into types is not possible. There is, however, more evidence for a constancy of character in the chimpanzee, in which each of the four phases of the cycle can be followed as readily as menstruation in the other species. Were it not for this fact, the characteristics of the cycle peculiar to individuals would have remained unobserved. Examples are cited in the section dealing with differences between individuals. At this point it is simply recalled that the length of the separate phases frequently varies less in individual adults than it does from animal to animal, and a certain constancy is exhibited in the extent to which the separate phases contribute to the total length of the cycle.

Within the cycle, variations in the phases are not correlated. This was not unexpected. Modifications in the preswelling phases induced by seasonal changes are not reflected by variations in the other phases. In addition, the lack of relationship between the length of the phases shown in table 9 was assumed to preclude the possibility of any correlation unless it were a negative one between the swelling and postswelling phases. This assumption was confirmed by a determination of the coefficient of correlation for each of the six possible combinations of phases. In the determination of the coefficient the mean length of one phase for each of the 22 subjects was taken as X and the mean length of the other as Y. N was 22 and the value of r to be significant should be 0.423 (Lindquist, 1940). In every case it was less: preswelling and swelling phases, $r = .223$; preswelling and postswelling phases, $r = -.095$; preswelling phase and menstruation, $r = -.009$; swelling and postswelling phases, $r = -.392$; swelling phase and menstruation, $r = .121$; postswelling phase and menstruation, $r = -.104$.

*Length of the phases and reproductive performance.* Two observations contribute to the conclusion that during the first months after the menarche some relationship exists between length of phases and reproductive performance, but variations in the length of the phases

which occurred later seem unrelated to the degree of fertility except as long cycles reduced the opportunity for conception. The latter part of this conclusion is essentially similar to Hartman's (1932) statement, " . . . monkeys may well be irregular and yet reproduce or be regular and remain sterile."

The first of the observations on the length of the phases and reproductive efficiency during adolescence is that, in 9 of 11 subjects which are known to have mated more than once between the time of the menarche and the first conception, there is a close correlation between the time of the first conception and the appearance of shorter preswelling and swelling phases and longer postswelling phases. The protocols of Wendy and Lita are given (tables 1 and 3) and in 7 other animals the correlation is as close. In the 2 subjects, Bentia and Mimi, in which the interval between the appearance of phases of average length and the first conception was considerable, secondary factors may have accounted for the delay.

The second observation bearing on the relationship between length of phases and reproductive performance requires explanation. Most of the breeding females were confined with males during the first cycles after the menarche when longer preswelling and swelling phases and shorter postswelling phases were being experienced and also during later cycles when shorter preswelling and swelling phases and longer postswelling phases were being shown. The data are not extensive, but it may be significant that the ratio of pairings with conception to pairings without conception during the first cycles when the length of the postswelling phase was 8 days or less, was 1 to about 6 (11 of 78 pairings were followed by pregnancy), whereas during the later cycles the ratio was 1 to about 2.33 (53 of 177 pairings were followed by pregnancy). In addition, the ratio of abortion within 100 days of conception during the first cycles was 1 to 1.75 (4 of 11 pregnancies), whereas it was 1 to 4.66 (9 of 51 pregnancies) during later cycles.

The conclusion, that the correlation between length of phases and reproductive performance tends to disappear after the first months, is the result of a comparison of the data in tables 9 and 10 with those in table 11. In the former tables the mean length of the phases is shown for each subject as well as the extent to which the separate phases contributed to the total length of the cycle. In table 11 the females which have been pregnant are arranged in the order of adjudged reproductive efficiency based on the ratio of normal pregnancies to abortions and stillbirths and on the ratio of fertile matings to cycles during which the females were confined with the males.

It is noted in passing that two measures of reproductive capacity are represented, but the correlation is fairly close. The ratio of fertile matings to cycles spent with the males is generally highest in females in which the ratio of normal pregnancies to abortions and stillbirths is also high.

Reference to the 4 animals, Wendy, Soda, Alpha and Josie, in which fertility was highest, to the 2 adolescent subjects, Bula and Lita in which the percentage of sterile matings was high, and to Lia and Mimi who never had a normal pregnancy, and in which the percentage of sterile matings was high, is sufficient to show that no correlation exists save in the postswelling phase in the adolescent subjects. The mean length of the preswelling phase is short in Alpha, Wendy and Bula and long in Lia and Mimi, but it is also long in Soda. The mean length of the swelling phase is shorter than the average in Lia and

TABLE 11. ARRANGEMENT OF SUBJECTS IN ADJUDGED ORDER OF REPRODUCTIVE EFFICIENCY

| Order | Subject | Normal Pregnancies | Abortions and Stillbirths | Cycles with Males | Fertile Matings |
|---|---|---|---|---|---|
| 1 | Wendy | 5 | 0 | 19 | 5 |
| 2 | Soda | 4 | 0 | 11 | 4 |
| 3 | Alpha | 3 | 0 | 7 | 3 |
| 4 | Josie | 4 | 1 | 11 | 5 |
| 5 | Nana | 5 | 2 | 13 | 7 |
| 6 | Bimba | 2 | 1 | 6 | 3 |
| 7 | Dita | 3 | 1 | 15 | 4 |
| 8 | Fifi | 3 | 1 | 22 | 4 |
| 9 | Pati | 4 | 3 | 17 | 7 |
| 10 | Mona | 3 | 3 | 28 | 6 |
| 11 | May | 2 | 0 | 15 | 2 |
| 12 | Cuba | 3 | 1 | 32 | 4 |
| 13 | Bentia | 1 | 1 | 14 | 2 |
| 14 | Bula | 1 | 0 | 9 | 1 |
| 15 | Lita | 0 | 1 | 11 | 1 |
| 16 | Lia | 0 | 3 | 17 | 3 |
| 17 | Mimi | 0 | 2 | 16 | 2 |

longer in Mimi, despite the fact that their reproductive records were equally poor. A similar irregularity is seen among the 4 animals in which the reproductive performance was best. In the adolescent subjects, Bula and Lita, the postswelling phase was shorter than in any of the animals with the best reproductive records. On the other hand, the unusual length in Lia is a reminder that the short postswelling phase is not the only factor involved in low fertility. Menstruation in Mimi was generally longer than in any other member of the colony, but in Lia, whose reproductive record was also poor, it was short as it was in Josie, whose record was good. Similar irregularities are seen in the extent to which the separate phases contributed to the length of the cycle.

The same lack of correlation is seen when the two nonmating animals, Kambi and Nira, are compared with subjects in which the sexual behavior was normal. The postswelling phase was short in Kambi, but it was longer than in Lita who mated frequently. Nira's record, while not as regular as those of Wendy and Josie, was more so than those of Soda and Nana, and with respect to the length of the phases, showed none of the abnormalities characteristic of the record of Kambi.

*Temporal relationship between ovulation and detumescence.* Were the temporal relationship between ovulation and the beginning of detumescence known, not only would the significance of the changes described in the preceding sections be clarified, but the way would be prepared for a better understanding of the factors involved in the control of the reproductive cycle. If the relationship is constant and ovulation and detumescence coincide, as Zuckerman (1930, 1937) and Zuckerman and Parkes (1932) found in the baboon and in a single pig-tailed monkey, and as seems possible from the importance of progesterone for detumescence in the baboon (Gillman and Smyth, 1939; Gillman and Stein, 1941) and adolescent monkey (Hisaw, 1942), the variation in the length of the phases may be taken as a reflection of ovarian and gonadotropic activity. Presumably the prolonged swelling phase would indicate that ovulation is slow in occurring in the adolescent subject; the short postswelling phase would suggest that the stimulus to the formation and maintenance of the corpus luteum is less than in older animals. On the other hand, if ovulation and detumescence do not always coincide, as Elder and Yerkes (1936, b) concluded from the observation that some fertile matings occurred 6 days before the first signs of detumescence, the significance of the variation in the length of the phases is more obscure.

In an effort to resolve the question, the ovaries in 5 animals of known genital status were examined macroscopically following exposure by laparotomy. The mean length of the swelling phase for the subject Nira was $17 \pm 0.09$ days (table 9). At the time of the operation, the twelfth day after the beginning of tumescence, a single large follicle was seen, but there was no indication from its appearance that ovulation would have occurred within 2 or 3 days. The mean length of the swelling phase in a second subject, Fifi, was $15 \pm 0.12$ days. The ovaries were exposed early the fourteenth day and a large unruptured follicle was present. It is estimated ovulation would have occurred within 24 hours. The swelling phase of a third subject, Cuba, averaged $17 \pm 0.11$ days. The ovaries were removed about the middle of the fifteenth day. As in Fifi, a nearly mature follicle was present and it is estimated ovulation would have occurred soon. The fourth subject, Mimi, was hemi-oöphorectomized the evening of the day detumescence began. The appearance of the ruptured follicle gave every indication of a very recent ovulation. In the fifth animal, the adolescent female Lita, the relationship between ovulation and detumescence was different. The mean length of the swelling phase in the 27 cycles since the menarche was $21 \pm 0.68$ days. Laparotomy was performed the seventeenth day. Despite the fact that detumescence had not begun, a corpus luteum of recent origin was seen and it was estimated the follicle had ruptured 1 or 2 days previously.

Had observations been confined to the first four subjects, the conclusion would have been justified that ovulation shortly before detumescence is indicated, and that for most purposes these two events

of the cycle can be considered as coinciding. The exception provided by the fifth animal reveals, however, that this is not always true. At the same time, the length of the interval between ovulation and detumescence in this single adolescent subject should not be construed as evidence for the conclusion that the temporal relationship varies in fully mature adults. What was found could be a characteristic of the adolescent animal and not at all representative of the relationship in adults.

The best support for the suggestion that the relationship between ovulation and detumescence varies in adults comes from the observations by Elder and Yerkes (1936, b) and Elder (1938, a) that in several instances impregnation followed a single mating by as much as 6 days prior to the beginning of detumescence. A re-examination of the records from which their evidence was taken suggests that in the animals in which the interval between copulation and detumescence was recorded as 6 days, relaxation of the genital swelling began a day earlier than Elder and Yerkes supposed and therefore this interval should be reduced to 5 days. But even if this minor change is made, and if two subjects are eliminated in which copulation appears to have occurred the first or second day of detumescence, three cases remain in which normal pregnancies followed matings as long as 5 days before the beginning of detumescence. The animals were mature and there is nothing to indicate that short postswelling phases with which early ovulation might be associated were being shown. It could be postulated that chimpanzee spermatozoa remain viable longer than those from many mammals, but the numerous observations reviewed by Hartman (1939) render this suggestion improbable. The possibility must also be considered that during the final stages of follicular growth, copulation stimulates a slightly premature ovulation, but there is no proof for such an effect in any primate.

If the latter possibilities are discounted, the evidence would appear to be incontrovertible that in adults a considerable variation exists in the length of the interval between ovulation and detumescence. Nevertheless, the need for caution in making such an interpretation must be emphasized. Any great variation would indicate a more haphazard relationship between ovarian activity and regression of the genital swelling than is generally encountered in tissue responses involving the withdrawal of water (Astwood, 1939; Gillman, 1940). Not to be ignored either is the fact that the observations on the 4 adult subjects reported above indicated uniformity rather than variation in this relationship. The uniformity could be accounted for by the small size of the sample, but such a result would be surprising in view of the variation indicated by the Elder and Yerkes data.

The alternative hypothesis, that the relatively long interval between ovulation and detumescence in Lita is characteristic of adolescent rather than older subjects, is consistent with the observations

which are brought together in this article. The longer swelling phase and the shorter postswelling phase during the first months after the menarche in every subject suggest a basic difference between the period of adolescence and sexual maturity. That a shift of detumescence forward in the cycle toward ovulation may well be an expression of this difference is suggested by the fact that in the only adolescent animal which was available for examination, ovulation preceded detumescence by several days, whereas in four adult subjects the interval between detumescence and ovulation was very short. Because of the older observation that 3 of 12 single fertile matings were made the fifth day before detumescence began, this hypothesis must be offered for what it may stimulate in the way of further investigation rather than in any spirit of finality. At the same time, it is only the better part of caution to ask if the interpretation given the older data should not be re-examined in the light of the new. The technique of single matings may not be as decisive for the elucidation of the problem as was once thought.

*Age at menarche.* The age at the menarche is now known for 7 subjects and can be estimated for an additional 11. In members of the former group the mean was 8 years, 11 months, range 7 years, 4 months to 10 years, 2 months. This compares with a mean of 13.53 years in 250 girls, range 11.04 to 16.31 years (Engle and Shelesnyak, 1934). In the chimpanzees whose date of birth is unknown and for whom it was necessary to estimate the age of menarche, the mean is 8 years, 3 months, range 7 years to 9 years, 6 months.

Of the 18 subjects for whom the date of the first menstruation is known, or has been estimated with an acceptable degree of accuracy, 13 have been pregnant. Of these, the first pregnancy was normal in 7. In 2, Lita and Mamo, the first pregnancy was terminated by abortion about a month after conception. In Bimba abortion occurred after 42 days. In Bentia, Josie and Mimi miscarriage occurred the 224th, the 189th and the 167th day, respectively. Three of the 6 subjects (Bentia, Bimba and Josie) in which the first pregnancy was terminated by miscarriage have since had normal babies. One (Mimi) has had a second miscarriage, 1 (Mamo) was the subject from which a normal appearing 10.5-day embryo (Elder, Hartman and Heuser, 1938) and later a pathological embryo were removed, and 1 (Lita) had no opportunity for a second pregnancy.

*Period of adolescent sterility.* The term adolescent sterility has been used to designate the interval between the first menstruation and the time the female becomes capable of carrying the fetus to term (Hartman, 1931, 1932; Ashley-Montagu, 1939, a; Smith and Rubenstein, 1940). The period is not one during which conception is impossible. Several instances of first pregnancies terminated by abortion are cited by Ashley-Montagu and Smith and Rubenstein.

Thus far in the history of the colony at Orange Park there have

been 7 subjects (Alpha, Bula, Cuba, Dwina, May, Soda, Wendy) in which the first pregnancy was terminated by the delivery of a living infant and 6 in which the first pregnancy was terminated prematurely (Bimba, Josie, Lita, Mamo, Mimi) or by a stillbirth (Bentia) at the end of a gestation period of normal length (Nissen and Yerkes, 1943). If maturity is to be regarded as being attained when the subject first becomes capable of reproduction, determination of the length of the period of adolescent sterility must be confined to the 7 subjects in which the first pregnancies were normal. Data from the remaining 6 would not be comparable because maturity in the sense the term is used above had not been reached by the time of the first conception. We will explain below, however, that the members of the latter group may not be disregarded without leading to an unjustifiable weighting of the result.

In the 7 subjects in which the first pregnancy terminated normally the mean length of the interval between the menarche and the first conception is 11.33 months, the range 4 to 17 months. In the 6 subjects in which the first pregnancy was terminated by abortion or stillbirth the mean length of the interval between the menarche and the first conception is 2 years, 2 months and 22 days, range 7 months, 16 days to 5 years, 28 days.

Most of this difference is accounted for by the records of Bentia and Mimi in which the first fertile matings occurred 5 years, 1 month and 3 years, 9.5 months, respectively, after the menarche. In addition to the length of the sterile period, a peculiarity in these subjects is that the first conception did not coincide with the appearance of the longer postswelling phases. In Bentia postswelling phases of more than average length were displayed within 7 months after the menarche, but the first conception did not occur until 4.5 years later. In Mimi postswelling phases similar to those seen in the normal adult were being shown about 2 years after the menarche, but another year and a half elapsed before a mating was fertile. Because of this circumstance the prolonged period of sterility in these animals is not ascribed to factors related to adolescence. The explanation is believed to lie elsewhere.

The records of the other 4 animals in which the first pregnancy was terminated prematurely are not unlike those of the 7 subjects which gave birth to living infants. The mean length of the interval between the menarche and the first conception is longer, but the range of variation is not greatly different, and in all the correlation between the first conception and the appearance of the longer postswelling phases is close. We believe, therefore, that these subjects should be grouped with the 7 in which the first pregnancy was normal and in which the sterile period is attributed to adolescence.

When this is done the mean length of the period of adolescent sterility as given for the 7 subjects in which a close approximation

was possible must be revised upwards if our estimate is to be applicable to the group as a whole. The revision is necessary because the mean in the 7 subjects was only 11.33 months, whereas the mean length of the interval between the menarche and the first conception was 13.66 months in the 4 animals in which pregnancy was terminated prematurely. Were it possible to ascertain the time at which the members of this latter group first became capable of carrying a fetus to term, the mean would be still higher. The suggestion will be made, therefore, that the mean length of the period of adolescent sterility in the chimpanzees in the Orange Park colony is 1 to 1.5 years. If this estimate seems low, it must be recalled that of the 11 subjects for which there are data, 3 are known to have matured in less than a year, 5 in less than 1.5 years, and 2 (Josie and Mamo) might well have matured within 1.5 years, in the sense we have used this term, had the pregnancies terminated by abortion not intervened and prevented a determination of the point. Delay in the attainment of maturation beyond the second year is certain only in the case of Lita.

The estimate that the average length of the period of adolescent sterility is somewhat more than a year in the chimpanzee is not greatly different from that given by Hartman for the Rhesus monkey. Smith and Rubenstein state, however, that it may be considerably less than a year in this species. If so, and if the estimates given for the human female are correct, the chimpanzee is intermediate in position as it is with respect to the length of gestation and the time of maturity.

## DISCUSSION

The readily apparent genital swelling and menstruation in the chimpanzee have simplified the identification of the different phases of the reproductive cycle beyond anything that has thus far been possible in the Rhesus monkey or human female. Utilizing these endpoints in an analysis of data which have accumulated during the past 11 years, we have become aware of an unexpected degree of lability in the cycle. Fluctuation and change is the rule, especially in young animals during the first years after the menarche. Irregularities which often led to exclusion of the data from such cycles in earlier studies can to a very considerable extent be accounted for. Most are the consequence of stimuli having an internal or environmental origin and their loci in the cycle, almost without exception, can easily be ascertained.

The most easily identified factors influencing the character of the cycle are age, pregnancy and season. It is assumed that certain illnesses such as the wasting diseases and intoxications and malnutrition would have such an effect, but the colony has been singularly free from illness of this type so we have no information on which a positive statement could be based. Gastrointestinal disturbances and colds may have an effect, but if so, it is so closely associated with the influ-

ence of season that the irregularities may not be ascribed with certainty to these illnesses. The recurrent variations which have been attributed to differences between individuals are striking, but until we know something about the nature of these differences, the sort of analysis that was profitable in the case of such dichotomies as adolescence and maturity, fertile and sterile animals, mating and nonmating types, winter season and summer season, illness and full physical vigor, will not be possible.

Less satisfying than the result of our effort to identify factors which influence the character of the cycle was the attempt to locate the site of action of such factors as age, pregnancy and season. They could modify the gonadotropic action of the pituitary, decrease the resistance of the ovary to pituitary stimulation, alter the sensitivity of tissues such as the uterus or perineum to estrogenic or luteal stimulation, or exert a general effect involving all three.

As far as the changes associated with age are concerned, a general effect rather than the first three more restricted possibilities seems more likely. The numerous observations bearing on the activity of the pituitary are summarized by Smith (1939) who cautions against placing too much dependence on assays of gonadotropic content of the pituitary. He states, however, "So far as the data permit generalization, it appears that gonadotropin begins to appear in the hypophysis of mammals during foetal life and gradually increases in amount after birth." Confirmatory evidence is given by Witschi and Riley (1940) following assays by a method which appears to be free from the objectionable features enumerated by Smith.

Evidence that a gradually developing sensitivity of the reproductive system to hormonal stimuli may also be involved is suggested *a*) by the determination that the ovary of the rat, rabbit, opossum and monkey is not responsive to gonadotropic stimulation until several days or weeks after birth (Corey, 1930; Hertz and Hisaw, 1934; Engle, 1936; Moore and Morgan, 1943), and *b*) by the work of Allen, Diddle, Burford and Gardner (1936) who showed that the estrogen threshold for menstruation in the immature and adolescent Rhesus monkey is higher than that in mature animals. Among other lower mammals examples of an increase in tissue sensitivity as animals mature are cited by Allen (1928), Allen, Hisaw and Gardner (1939) and Young (1941). On the other hand, Elder (1938, b) does not report any difference in the response of two prepuberal chimpanzees to the injection of estrone, despite an age difference of more than 4 years.

Observations on the chimpanzee supplementary to those reviewed above are of interest because of their bearing on the changes in the postswelling phase during the first months after the menarche. It will be recalled that in every subject the lengthening of the postswelling phase coincided with the shortening of the swelling phase. Examina-

tion of the ovaries in the adolescent subject Lita further revealed that ovulation can occur during at least a part of the period of short postswelling phases. In addition, there is evidence for the belief that the lengthening of the postswelling phase is a consequence of a delay in the beginning of detumescence rather than of a retardation of ovulation. It will be recalled, finally, that the frequency of early abortions was greater when the short postswelling phases were being shown.

These observations suggest that, in the postswelling phase during adolescence, there is not necessarily any sluggishness in the ovary as far as ovulation is concerned, and that in the corpora lutea which form during this period, the secretion of progesterone is not always adequate to cause a rapid detumescence or to support pregnancy. The deficiency of progesterone is suggested as being due to one or two circumstances. This hormone may be present, but the sensitivity of the tissues could depend on the same step-like preparation that Hartman has postulated for the estrogens and it may not therefore be completely effective. The alternative possibility is that the first corpora lutea do not function strongly and that there is an insufficiency of progesterone. The latter suggestion involves the idea that during much of adolescence luteinizing hormone action is sufficient to cause ovulation, but that the third gonadotropin, luteotropin (Astwood and Fevold, 1939; Astwood, 1941) comes in slowly and, until a certain amount is available, progesterone secretion is inadequate, resulting in a delay in detumescence and a high frequency of abortion. This hypothesis presents the difficulty that it does not account for the constant relationship between ovulation and menstruation; current theories of menstruation lead us to believe that a deficiency of progesterone would also be reflected in the time of bleeding. We are indebted to Dr. George W. Corner for the suggestion made during an informal discussion of the problem, that following ovulation the corpus luteum may perhaps run its normal course and secrete enough estrogen to delay menstruation until the corpus luteum begins to degenerate, while not secreting enough progesterone to overcome the effects of estrogen and thus to bring on the postswelling phase, promptly after ovulation.

Information that would enable us to account for the longer first menstrual periods is even less satisfying than that bearing on the changes in the other phases. The condition may be comparable with the menorrhagia or metropathia hemorrhagica frequently seen in the pubescent human female, but the etiology of this abnormality is uncertain (Fluhmann, 1939). The view is held by some clinicians that the profuse or prolonged puberal bleeding is associated with a failure of ovulation. Hartman states, however, that although the difference is slight, by and large the menstrual flow in monkeys is more abundant in winter than during the summer season of anovulatory cycles. Further uncertainty is added by the circumstance that although indi-

cations are that the first cycles in the chimpanzee are anovulatory, proof is still lacking.

When the considerations reviewed above are seen in perspective it is obvious that the most we can contribute to the subject of adolescence is a redefinition of the problem in the light of information which has accumulated since Hartman first proposed his 'staircase' theory. What lies behind the many changes is still problematical. We have cited evidence for the view that in the chimpanzee the shortening of the preswelling and swelling phases and the lengthening of the postswelling phase may be a consequence of an increased gonadotrpic activity coupled with a simultaneous increase in sensitivity of the tissues to hormonal stimuli. It must be borne in mind, however, that most of the data which support this suggestion were obtained from lower mammals and from animals much younger relatively than the chimpanzees used in this study. Furthermore, if these changes are attributable to increased gonadotropic activity in combination with a heightened sensitivity of the tissues, there is no basis for estimating the extent to which each is a contributing factor. It may well be that at one age changes in tissue sensitivity are more important and that at another age, such as the period of adolescence, maturation changes in the pituitary are more important. Equally puzzling is the nature of ovarian activity during the months between puberty and sexual maturation. Heretofore this period has been regarded as one of anovulatory cycles in the human female as well as in the monkey. The possibility that the luteal phase might also undergo a step-like development does not seem to have been suggested. Our observations on the chimpanzee are not numerous by the standards which are possible when the lower primates are used, but they are consistent in suggesting that during much of adolescence the failure is one of luteal function rather than of ovulation.

No attempt has been made to account for the temporary reversion after parturition to the adolescent condition beyond the suggestion that, following the interval of placental activity during pregnancy (Newton, 1939), the pituitary passes through a short period of adjustment comparable with the maturation of adolescence. Regardless of the merit of this suggestion, the similar character of the cycle following pregnancy and during adolescence provides a sufficient reason for believing that as more is learned about the process of sexual maturation, the nature of the changes following pregnancy will also be clarified.

The problem of the influence of seasonal factors on the character of the cycle would be thought to be less complicated than that of the effect of age, if for no other reason than that only the preswelling phase is affected, but we cannot be sure this is true. There is nothing in the numerous investigations reviewed by Bissonnette (1936),

Marshall (1936) or in the experiments reported by Dempsey and Searles (1943) to indicate that stimuli of light and temperature have any other effect than that mediated by the pituitary. Data obtained from the hedgehog, ground squirrel and sheep suggest even more strongly that the quiescence of the reproductive system during the winter anestrum is due to a decreased activity of the pituitary rather than to any change in responsiveness of the ovaries or changes in the sensitivity of the secondary tissues to estrogenic stimulation. The gonadotropic potency of the pituitaries removed during the anestrum was low and no difficulty seems to have been encountered in stimulating either the ovaries or the other genital tissues by appropriate substitution therapies (Courrier, 1924; Allanson and Deanesly, 1934; Foster, 1934; Moore, Simmons, Wells, Zalesky and Nelson, 1934; Cole and Miller, 1935). Engle and Shelesnyak state, however, that in their monkey colony the only failures in inducing ovarian activation with extract of the anterior pituitary gland occurred during the summer months. This experience compels us to reserve any conclusion that the periods of genital quiescence frequently seen in the chimpanzee in winter are due to the failure of the pituitary to initiate follicular growth.

Thus far in the presentation of data and discussion of the subject we have been concerned principally with the chimpanzee. In general reference has been made to data from the other primates only when they have been suggestive for the problem of cyclic reproductive activity in the chimpanzee. Of interest now is the extent to which similarities and differences are shown by other primates, including man.

The irregularity and greater length of the first cycles have been noted in the Rhesus monkey (Corner, 1923; Hartman, 1928, 1932; Smith and Rubenstein, 1940), the baboon (Zuckerman, 1930) and the human female (Novak, 1921; Engle and Shelesnyak, 1934; Knaus, 1935; Arey, 1939). Another mark of irregularity in the first cycles in the human female is the longer and frequently more profuse menstrual bleeding (Fluhmann, 1939). The implication is contained in the Hartman (1932) article that this may also be true in the monkey. The addition of the chimpanzee to the list of species displaying these irregularities strengthens the opinion that such cycles are characteristic of the adolescent primate.

Hartman (1932) states that when a female resumes cyclic changes after lactation or the summer anestrum she "gets her stride" gradually, as during adolescence. The possibility of irregularities in the cycle of the baboon after an interval of lactation is suggested by Zuckerman and Parkes (1932). Latz and Reiner (1935) state that about 50 per cent of women menstruate irregularly after childbirth, although the percentage is smaller in the 24 cases cited in their table 1. A lack

of correlation between the duration of the menstrual flow and the length of the cycle is seen in the chimpanzee, the monkey (Allen, 1927) and in man (Fluhmann, 1934).

Differences in the 4 primates under discussion appear in the length of the cycle, the age at the menarche and in the length of the period of adolescent sterility, but none is suggestive of a very fundamental difference between the species. Much more interesting, therefore, are the differences in the nature and mode of action of the seasonal factors.

The question of the influence of season has been argued back and forth and is reviewed by Zuckerman (1930, 1931), Engle and Shelesnyak (1934), Whitaker (1938) and Ashley-Montagu (1939, b). It seems true, as Zuckerman emphasizes, that infants have been born at all times of the year in most if not in all of the primates. Nevertheless, conception is most frequent at certain seasons in the lemur (van Herwerden, 1925) and in several of the monkeys (Heape, 1897; van Herwerden, 1906; Corner, 1932; Hartman, 1932). In other primates quite a different manifestation of seasonal influence is shown such as the seasonal amenorrhea in the baboon (Gillman, 1935), man (Engle and Shelesnyak, 1934) and the chimpanzee. The particular contribution of our data from the chimpanzee is that they serve to set off certain similarities and differences which have not hitherto been emphasized.

A first conspicuous difference is that in the lemur and monkeys the effect seems to be exerted toward the end of the follicular phase in such a way that ovulation does not occur. In the baboon and chimpanzee evidence from the condition of the genital swelling indicates the failure is one of follicular development rather than of ovulation. The temporary amenorrhea in the cases cited by Engle and Shelesnyak (1934) suggests that the human female resembles the baboon and chimpanzee in this respect. Another difference between the two groups is that the seasonal anovulatory cycles of the monkeys do not seem to be correlated with age, whereas the seasonal amenorrhea of the chimpanzee and man is most common during adolescence.

Within the group composed of the baboon, the chimpanzee and the human female, a further interesting difference is shown. Prolonged cycles in young girls are more frequent during the summer months at latitude 41°N. (Engle and Shelesnyak, 1934) whereas similar irregularities of the cycle in the baboon at 34° latitude S., (Gillman, 1935) and chimpanzee are more frequent during the winter months at 30° latitude N. The human female and chimpanzee are alike, however, in that the incidence of the menarche is greatest in the season which is most favorable for normal cyclic activity.

A perplexing problem that may resolve itself into a species difference arises from a comparison of the variation in length of the preovulatory and postovulatory moieties of the cycle. Of those who have

studied primates, Hartman (1933) and Fluhmann (1939) appear to be the only investigators who state that the preovulatory interval is more constant than the postovulatory interval. Hartman's observations on the Rhesus monkey have recently been confirmed by Corner (personal communication). In three other species, *Papio hamadryas*, *Macaca nemestrina* (Zuckerman, 1936, 1937; Zuckerman and Parkes, 1932) and the chimpanzee (Elder, 1938, a; Elder and Yerkes, 1936, b; fig. 2 in this study) and in the human female (Ogino, 1932; Knaus, 1934) the preovulatory phase is thought to be the more variable.

Quite apart from the confirmation given by Corner, any question of the accuracy of Hartman's method for determining the time of ovulation would appear to be settled by a comparison of the embryos whose ages were estimated in this way (Lewis and Hartman, 1933; Heuser and Streeter, 1941), although in the appendix of the latter publication reference is made to "error in determining the ovulation age" and an adjustment according to "the amount of development." In species with pronounced genital swellings estimation of the time of ovulation by reference to the beginning of detumescence is also thought to be reliable. Gillman (1935, 1937) states there is evidence at hand to show that the temporary decrease in the diameters of the perineum 24 to 72 hours before the attainment of maximum swelling by *Papio porcarius* may be due to ovulation. In adults of other species not less than 13 pairs of ovaries have been examined about the time of maximum swelling or very shortly after the beginning of deturgescence: 7 Hamadryas baboons, 1 Chacma baboon and 1 pigtailed monkey, by Zuckerman and Parkes, and 4 chimpanzees by us. In only 1 adolescent chimpanzee did ovulation precede detumescence by any considerable interval. On the basis of existing information, therefore, the difference in variability of the preovulatory and postovulatory periods in the Rhesus monkey (fig. 4 in Hartman, 1933), on the one hand, and the baboon and chimpanzee (fig. 2 in this article), on the other, may not reasonably be ascribed to the methods of investigation. Something else must be involved, but until additional information can be obtained, we are more or less forced to think in terms of a species difference.

## SUMMARY

An investigation of the influence of age, pregnancy, season, illness and social relations on the character of the reproductive cycle in the chimpanzee was based on the records of 653 cycles and the opportunity to examine the ovaries in 5 subjects. The study was facilitated by the division of the cycle into 4 easily identified parts or phases of genital reaction: a preswelling phase or short initial period of ovarian follicular inactivity, a genital swelling phase corresponding in general to the follicular phase, a postswelling phase corresponding closely to the luteal phase, and menstruation. In adults ovulation is indicated

as occurring about 24 hours before the end of the genital swelling phase. In adolescent animals it may occur several days earlier.

In 7 animals of known age the mean age at the time of the menarche was 8 years, 11 months. During the first months after the menarche striking changes occurred in the character of the cycle. The preswelling and swelling phases and menstruation became shorter, the postswelling phase became longer. The mean length of the cycle was $50 \pm 4.38$ days during the first months after the menarche and $35 \pm 0.79$ days during the second half of the seventh year. The period of true adolescent sterility varied from about 4 months to more than 2 years; the mean length is estimated to be 1 to 1.5 years. Existing information, derived mostly from experiments on lower mammals, suggests that the changes associated with age are a consequence of increased gonadotropic activity by the pituitary and of a heightened sensitivity of the reproductive tissues to hormonal stimuli. Ovulation is believed to occur during much of adolescence and the relative sterility of the period is thought to be due to a deficiency of luteal function rather than to the failure of ovulation. After pregnancy in many animals there is a temporary reversion to the condition seen during adolescence.

In the winter months, particularly in younger animals, a prolonged preswelling phase resulted in a temporary amenorrhea. Any effect of the illnesses which have occurred is questionable because the lengthened preswelling phase could as well be a consequence of the winter season when most of the illness occurred. No part of the cycle was affected by isolation, copulation or the sex of the cage-mate. Among individuals persistent differences are seen, particularly in the length of the separate phases and in the extent to which the separate phases contribute to the total length of the cycle. Until our information is more complete it can only be supposed that these differences have as their basis deviations in the endocrine balance or other chronic peculiarities of function.

The irregularity of the cycle is regarded as an inevitable consequence of the interplay between the separate phases and the internal and environmental stimuli enumerated above. Most of the variation, however, is associated with variations in the genital preswelling and swelling phases. The postswelling phase and menstruation are the most stable. The conclusion that in the monkey the luteal phase is the most variable is discussed. Within the cycle fluctuations in the phases are not correlated. The poor reproductive performance during adolescence is associated with the short genital postswelling phase rather than the prolonged preswelling or swelling phase, but later variations in the length of the phases were unrelated to the degree of fertility except as long cycles reduced the opportunity for conception.

The reproductive cycles of the Rhesus monkey, baboon, chimpanzee and man are compared. The irregularities in the first cycles

after the menarche are common to all. The long cycle in the chimpanzee, compared with the cycle of the monkey and man, is a consequence of the longer interval between the end of menstruation and ovulation. The luteal phase and menstruation are shorter. Conspicuous differences are seen in the influence of season. In the lemur and monkey the effect is exerted toward the end of the follicular phase and ovulation does not occur. In the baboon and chimpanzee, and presumably in man, the failure appears to be in the initiation of follicular development and is confined largely to younger animals. The seasonal amenorrhea in the baboon and chimpanzee occurs during the winter months; that in the pubescent human female during the summer months.

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# EFFECT OF THYROIDECTOMY ON RESISTANCE TO LOW ENVIRONMENTAL TEMPERATURE[1]

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FROM the evidence available in the literature, it is difficult to estimate the rôle of the thyroid gland in the resistance of animals to cold. Removal of this gland was found to interfere with the regulation of body temperature on exposure to cold (Boldyreff, 1913; Cori, 1922; Shenck, 1922; Pfeiffer, 1923; Abderhalden, 1925; Korenchevsky, 1926; Wadi, 1927; Fetscherin, 1934; Ring, 1939), although contradictory results have been reported (Isenschmid, 1922, 1923). On the other hand, the increased amount of heat produced by animals kept at low temperatures was not affected by thyroid removal (Hildebrandt, 1921; Grafe and von Redwitz, 1922; Isenschmid, 1923; Collip and Billingsley, 1936; Ring, 1936) or only slightly (Ring, 1939, 1942), so that some investigators have questioned the importance of this gland in the response to cold (Hildebrandt, 1921; Isenschmid, 1923). Incidental mention of shorter survival in the cold after thyroidectomy has also been made in the literature (Pfeiffer, 1923; Korenchevsky, 1926; Ring, 1939); but no systematic study of this problem has been made. It was therefore decided to compare the survival of normal and thyroidectomized rats exposed to a low temperature. This procedure showed that thyroidectomy decreased the resistance of rats to a low temperature, but that various factors could modify the extent of this decrease in resistance.

## METHODS

Albino rats fed on standard food (Purina Fox Chow) were thyroidectomized and, 2 weeks later, placed in an unlighted room at a temperature of 0 to 2°C. The food consumption of the animals was measured for 3 days previous to, and for the 3 first days during cold treatment. Animals gaining more than 1 gm. daily, on the average, in the 2 weeks following thyroid extirpation were eliminated. In the animals used, careful examination at autopsy failed to reveal visible thyroid fragments.

Substitution therapy was attempted in some thyroidectomized animals by injections of 0.5 mg. of thyroxine twice daily in oily suspension, beginning on the day before exposure to cold. As a further control, animals deprived of the parathyroid glands alone were also tested in the cold, since thyroidectomy in the rat includes removal of the parathyroids.

The influence of the time interval between thyroidectomy and the exposure to cold was estimated by comparing the reaction of male rats weighing 130 to 180 gm. operated either 2 weeks or 6 to 12 hours before the exposure to cold. A few of the animals operated 6 to 12 hours prior to exposure died

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[1] A preliminary report of this work was presented before the American Association of Zoologists (Leblond and Gross, 1942).

during the first day of exposure. Since these deaths were considered to be due to operation they were not recorded in figure 1.

The influence of the surrounding temperature previous to the exposure to cold was tested by comparing thyroidectomized rats kept for a week at 33 to 34°C. with some kept either at room temperature (19–24°C.) or in a ventilated oven adjusted at 25°C. Some of the rats in these groups were operated 4 weeks before cold treatment. It may be noted that rats appeared to live in good condition for indefinite periods of time at 33 to 34°C.

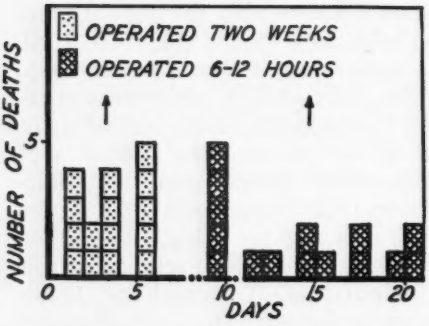


FIG. 1. Survival (in days) of rats exposed to a temperature of 0 to 2°C. beginning either 2 weeks or 6 to 12 hours after thyroidectomy. The arrows indicate the averages. Each rectangle represents data for one rat.

Some thyroidectomized rats were gradually adapted to a low temperature by placing the animals in the cold room for one hour the first day, 2 hours the second day and so on up to 5 hours on the fifth day, while on the 6th day the animals were permanently left in the cold.

Finally, in view of the importance recently attributed to the adrenal glands in resistance to cold, adrenal weights were recorded in thyroidectomized animals exposed to cold under various conditions (table 2). The figures in brackets after the adrenal weights refer to the extreme variations.

## RESULTS

At 0 to 2°C., normal adult rats usually did not die for several weeks or months. In the preliminary experiment (table 1), while the controls survived 20 days, at which time the experiment was interrupted, all thyroidectomized rats died within a week. Thyroxine treatment kept all but one of 8 thyroidectomized animals alive, in good condition and gaining weight. Removal of the parathyroids alone did not affect the resistance of the animals to cold. In the following experiments, it was found that of a total of 108 thyroidectomized rats exposed to cold, all died but 7, which survived for over 3 weeks. Since in 4 of these animals visible thyroid remnants were found at autopsy, it was assumed that the 3 other survivors had also retained some thyroid tissue. The data for these 7 animals were not included in the averages.

The thyroidectomized rats ate less food at room temperature than the normal ones (table 1). However, the amount consumed in the cold did not differ significantly in the three groups examined.

When removal of the thyroid was performed just before exposure to cold (fig. 1), the survival was considerably prolonged. It was 14.7 days on the average, while the controls operated two weeks previous to the cold treatment died in an average of 3.3 days.

When the thyroidectomized rats were habituated to a temperature of 33 to 34°C. for a week, they had little resistance to the cold (fig. 2), and died in an average of 1.7 days; the controls survived for 3.6

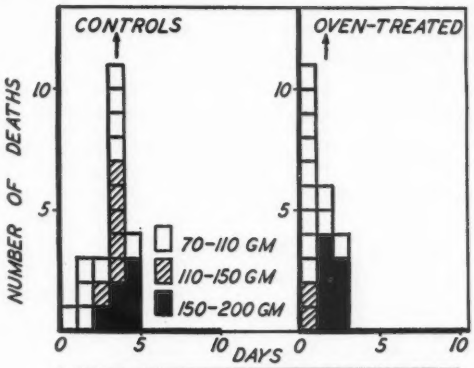


FIG. 2 (*upper*). Survival (in days) of rats exposed to a temperature of 0 to 2°C. after being kept either at room temperature (controls) or at 33 to 34°C. (over-treated). Each rectangle represents data for one rat.

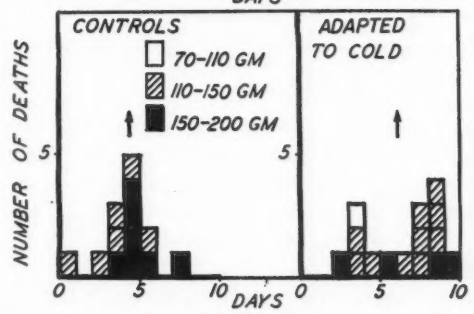


FIG. 3 (*lower*). Survival (in days) of rats exposed to a temperature of 0 to 2°C. after they were kept either at room temperature (controls) or gradually adapted to cold. Each rectangle represents data for one rat.

days. Groups of animals of various weights were used; in every weight group the heat-treated animals died sooner than the controls at room temperature. This experiment also showed that in thyroidectomized rats the heavier the animal, the longer was the survival in the cold.

Animals slowly adapted to low temperatures (fig. 3) showed an average survival of 6.9 days, while the controls died within an average of 4.4 days.

Finally, examination of the adrenal weights after thyroid removal showed that thyroidectomy did not prevent, although it reduced, the weight increase of the adrenals in animals exposed to cold. The results in sections 2 and 3 of the table must be interpreted with caution as the adrenal weights in the three groups listed last were obtained from dead animals. It seems that in the three sections the less resistant animals had smaller adrenals.

## DISCUSSION

The importance of the thyroid gland in resistance to cold was confirmed by the finding that thyroidectomized rats died rapidly at 0 to 2°C. unless they received injections of thyroxine (table 1). Nevertheless, thyroidectomized rats like normal rats were able to increase their metabolism in the cold, as shown by measurements of food consumption (table 1) and heat production (Hildebrandt, 1921; Collip and Billingsley, 1936; Ring, 1936). Ring found that the basal (1939) and the maximal (1942) metabolism could be slightly lower in thyroidectomized than in normal animals on exposure to colds, but was reluctant to explain the rôle of the thyroid on the basis of this difference (1942). Since in our experience the thyroidectomized rats lived

TABLE 1. INFLUENCE OF THYROIDECTOMY ON THE SURVIVAL OF ADULT FEMALE RATS EXPOSED TO A LOW TEMPERATURE

| Groups of Animals[1] | Survival | Average Daily Food Consumption | | Average Changes in Body Weight |
|---|---|---|---|---|
| | | Room Temperature | 0 to 2°C. | |
| | *days* | *gm.* | *gm.* | *gm.* |
| Normal | >20 | 10.2 | 17.6 | + 1 |
| Thyroidectomized | 3 to 6 | 8.8 | 17.0 | |
| Thyroidectomized & thyroxin-treated | >20 | 8.4 | 17.5 | +14 |
| Parathyroidectomized | >20 | | | + 5 |

[1] Eight animals per group.

in the cold for one or several days in good condition and then became moribund and died, it would appear that the high level of metabolism necessary for resisting cold could be reached, but not maintained for more than a few days in thyroidectomized animals.

The following influences were found to modify the resistance of thyroidectomized rats exposed to a low environmental temperature: *a*) the body weight of the animals (fig. 2); *b*) the time interval between operation and exposure to cold (fig. 1), as probably some thyroid hormone was retained in the tissues for a time after thyroidectomy and could eventually facilitate both resistance and adaptation to cold; *c*) the temperature to which the animals were submitted before operation (fig. 2 and 3). The decreased resistance to cold in the case of animals previously kept at 33 to 34°C. might be the consequence of the low metabolism obtained upon exposure at a high temperature (Benedict and MacLeod, 1929; Schwabe, Emery and Griffith, 1938; Swift and Forbes, 1939; Black and Swift, 1943). The lower basal metabolism in such animals when exposed to cold could be a handicap in itself; or it might act by decreasing the weight of the adrenals (table 2).

Examination of the adrenal weights revealed smaller adrenals in the less resistant groups in three experiments (table 2). This might be a coincidence or, since emphasis has been placed on both the cortex

TABLE 2. ADRENAL WEIGHTS OF THYROIDECTOMIZED RATS EXPOSED TO A LOW TEMPERATURE

| Groups of Animals | Number | Time Interval Between Operation and Exposure to Cold | Average Survival, days | Adrenal Weights, mg. |
|---|---|---|---|---|
| 1. *Effect of exposure to cold for 3 days on adrenal weights of 150–180 gm. male rats after thyroidectomy* | | | | |
| Normal | 6 | | > 3 | 32 (27–38) |
| Normal, cold | 6 | | > 3 | 41 (36–50) |
| Thyroidectomized | 6 | | > 3 | 29 (26–37) |
| Thyroidectomized, cold | 5 | 14 days | > 3 | 35 (30–43) |
| 2. *Effect of exposure to cold for 10 days on adrenal weights of adult female rats at various intervals after thyroidectomy* | | | | |
| Normal | 6 | | >10 | 45 (35–56) |
| Normal, cold | 6 | | >10 | 52 (32–64) |
| Thyroidectomized, cold | 5 | 6 hours | >10 | 52 (36–67) |
| Thyroidectomized, cold | 8 | 14 days | 5 | 41 (35–53) |
| 3. *Effect of exposure for 2 to 3 days on adrenal weights of young thyroidectomized female rats exposed to cold, with or without pretreatment at 34°C.* | | | | |
| Thyroidectomized, cold | 6 | 29 days | 3 | 18 (14–24) |
| Thyroidectomized, pretreated at 34°C., cold | 6 | 29 days | 2 | 13 (11–19) |

(Tyslowitz and Astwood, 1942) and the medulla (Cannon, 1932; Ring, 1942) of the adrenal as factors in the resistance to cold, the adrenal gland might really be instrumental in producing the variations in survival time found in thyroidectomized animals. Before discussing this problem, it must be noted that the behavior of adrenalectomized and thyroidectomized rats in the cold was quite different. Untreated adrenalectomized rats rapidly showed a deteriorating condition and death in the cold (Selye, 1937; Tyslowitz and Astwood, 1942), while death occurred in thyroidectomized rats after a few days during which the animals remained in good condition. Furthermore, Selye found that once rats had been adapted to cold, removal of the adrenals no longer affected the resistance to cold (1937). Similarly, unpublished experiments showed us that adrenalectomized rats given a 1 per cent sodium chloride solution as drinking water could survive for several weeks in the cold, provided they were given desoxycorticosterone[2] for the first few days of exposure (5 mg. daily in 2 injections). This experiment indicates that, except at the beginning of the exposure to cold, the adrenal hormones were unnecessary or at least could play only a minor rôle in the resistance to cold. On the other hand, the amount of adaptation to cold which may be obtained after thyroidectomy was limited, since death was delayed only by 2.5 days on the average (fig. 3). In any event, the consistent inability of thyroidectomized animals to survive in the cold showed that the thyroid hormone, in contrast to the adrenal hormones, was indispensable for resistance to

[2] Desoxycorticosterone (Percorten), Ciba Pharmaceutical Products, Inc.

cold. Therefore, the rôle played by the thyroid in resistance to cold could not be attributed to changes in adrenal function.

## CONCLUSIONS

The rôle of the thyroid hormone in the resistance to cold is confirmed by the observation that adult rats thyroparathyroidectomized two weeks previously die within a week at 0 to 2°C. while normal, parathyroidectomized, and thyroxine-treated thyroidectomized rats survive for several weeks at the same temperatures (table 1).

However, the death of thyroidectomized animals in the cold occurs only after an initial period of one to several days during which the general appearance and food consumption of the animals are comparable to those of normal controls. Hence, in contrast with the adrenals, the thyroid gland plays a small rôle if any in the first reactions following exposure to cold (such as the increase in metabolism, in food consumption and in adrenal weight).

The resistance of thyroidectomized rats on exposure to cold is affected by: *a*) the time interval between operation and exposure to cold, as the sooner the animals are placed at 0 to 2°C. after operation the longer they survive (fig. 1); *b*) the body weight, as larger animals are more resistant (fig. 2), and *c*) the environmental temperature of the surroundings before exposure to cold, as a previous exposure at 33 to 34°C. shortens, and a previous adaptation to cold lengthens the survival of thyroidectomized rats to 0 to 2°C. (fig. 2 and 3).

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# STUDIES ON THE RESPONSE OF HYPOPHYSECTOMIZED RATS TO INTRAPERITONEAL GLUCOSE INJECTIONS[1,2]

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SEVERE disturbances in the electrolyte and water distribution of the body can be induced by the injection of glucose solutions intraperitoneally (Schechter, *et al.*, 1933; Darrow and Yannet, 1935, 1936). The relation of the adrenal cortical hormones to this response has been extensively studied (Gilman, 1934; Swingle, *et al.*, 1936; Robinson and Hegnauer, 1936; Gaunt, Remington and Schweizer, 1937; Harrison and Darrow, 1938; Remington, 1940, 1943; Cantarow and Rykoff, 1940; Wyman and tum Suden, 1942).

Gaunt, Remington and Schweizer (1937) reported that in certain respects hypophysectomized rats differed from both normal and adrenalectomized animals in their response to intraperitoneal glucose. The experiments reported here were undertaken in an attempt to make a more complete analysis of the nature and cause of this difference.

## METHODS

Experiments were conducted 5 to 10 days after hypophysectomy in adult male rats weighing 150 to 200 gm. After an 18-hour fast, each animal was injected with 10 per cent of its body weight of a 5.5 per cent solution of glucose. The animals were kept in metabolism cages until they were killed. Determinations were made at intervals of 3, 6, and 12 hours after glucose injection.

Specific gravity of the whole blood was determined with a falling drop apparatus (Fisher), and hemoglobin was measured in a photoelectric hemometer. All blood figures are on samples drawn from the heart unless otherwise stated. The volume of ascitic fluid was measured by free drainage from a median ventral abdominal incision. The protein content of the ascitic fluid was calculated from its specific gravity, after the method of Weech, *et al.* (1936) for plasma proteins. Glucose in the ascitic fluid was determined with

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[2] These data are taken from a thesis by S. J. accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy by the Graduate School of Arts and Science, New York University.

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Benedict's quantitative solution, and chloride by the Volhard method for urine chlorides.

Blood pressure readings were made without anesthesia in an apparatus described by Duncan, Hyman and Chambers (1943), which employs microscopic observation of blood flow in the arterioles of the interdigital web of the hind toes. This method in our hands, gives pressure figures somewhat lower than by the method of Williams, Harrison and Grollman (1939), but the readings were consistent and in all cases checked within 5 mm. Hg. by two observers.

Six adrenalectomized animals were included in this investigation in order to obtain data, particularly on blood pressure, not already supplied by Remington (1940, 1943). These animals received glucose injections 18 hours after adrenalectomy.

One series of hypophysectomized rats was treated with adrenal cortical hormones before and after receiving glucose injections. Since maximal effects were sought, both desoxycorticosterone acetate and adrenal cortical extract were given. Two and one-half mg. of desoxycorticosterone acetate[4] was injected at the beginning of the pre-experimental fast, and another 2.5 mg. at the time the glucose was administered. A total of 6 cc. of cortical extract was injected in 6 does at 2-hour intervals beginning at the time glucose was given.

One series of animals received 6 cc. of 0.9 per cent NaCl in 6 doses at 2-hour intervals beginning at the time of the glucose injection, to observe the effect on the volume of ascitic fluid.

## RESULTS

*Survival.* Forty per cent of the untreated hypophysectomized animals died 8 to 10 hours after glucose injection. The condition of the remaining animals when killed at 12 hours was such that it is doubtful that any could have survived. All normal and hypophysectomized animals receiving cortical hormones survived the 12-hour experimental period and, judged by their general condition, would have lived indefinitely. The administration of large quantities (10 cc.) of normal saline solution maintained life in 5 hypophysectomized animals after injections of glucose, but survival figures only are available on these cases.

*Urine excretion.* Exact measures of urine volume were not obtained in all cases, since portions of the samples were often lost while blood pressure readings were being taken. The following generalizations can nevertheless be made.

All animals were anuric up to 6 hours following glucose injection. Urine excretion began shortly after that time in normal intact animals, and by 12 hours more than one-third of the injected fluid could be accounted for as urine.

Urination was observed only rarely, in very scanty amounts, in untreated hypophysectomized rats. Hypophysectomized animals

[4] We are indebted to Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Inc., for the desoxycorticosterone acetate used here.

given cortical extract excreted more urine than the untreated hypophysectomized group, but less than the normal control animals. Urine excretion in normal animals could generally be correlated with the amount of fluid absorbed from the peritoneal cavity, but this was not the case in other groups.

*Volume of peritoneal fluid* (*table 1, column 1*). In normal animals

TABLE 1. AVERAGE FIGURES ON THE RESPONSE OF NORMAL, HYPOPHYSECTOMIZED AND ADRENALECTOMIZED RATS TO THE INTRAPERITONEAL INJECTION (10 CC. PER 100 GM. BODY WEIGHT) OF ISOTONIC SOLUTIONS OF GLUCOSE

| | | 1 | 2a | 2b | 3 | 4 | 5 | 6a | 6b | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | No. Animals | Ascitic Fluid Recovered, cc./100 gm. body wt. | Glucose in Ascitic Fluid, mg. % | Glucose Absorbed gm./100 gm. body wt./hr. | Chloride in Ascitic Fluid m. eq./l | Estimated Protein of Ascitic Fluid gm. % | % Change in Hemoglobin | Specific Gravity of Whole Blood | % Change in Blood Specific Gravity[2] | Blood Pressure mm. Hg | Rectal Temperature 0° F. |
| *Before injection of glucose solution* | | | | | | | | | | | |
| Normal | 35 | | | | | | | 1.0627 | | 90 | 101 |
| Hypx. | 33 | | | | | | | 1.0606 | | 79 | 98 |
| *Three hours after injection of glucose solution* | | | | | | | | | | | |
| Normal untreated | 15 | 14.2 | 149 | 0.11 | 75.5 | | | 1.0704 | +12.2 | 65 | 94.8 (8) |
| Hypx. untreated | 14 | 13.6 | 159 | 0.11 | 75.4 | | +17.0 | 1.0671 | +10.8 | 49 (8) | 91.0 (9) |
| Hypx.-cortical hormone | 8 | | | | | | | | | 67 | 94.0 |
| Adrenalx. | 6 | | | | | | | | | 48 | 91.5 |
| *Six hours after injection of glucose solution* | | | | | | | | | | | |
| Normal untreated | 13 | 13.2 | 102 | 0.07 | 78.0 | 1.12 | +10.0 | 1.0693 | +10.7 | 70 (10) | 96.0 (10) |
| Hypx. untreated | 14 | 13.0 | 113 | 0.07 | 77.3 | 1.11 | +29.0 | 1.0665 | +9.7 | 60[3] (4) | 87.6 (10) |
| Hypx.-cortical hormone | 8 | | | | | | | | | 68 | 94.6 |
| Adrenalx.[1] | 6 | 8.2 | 76 | 0.08 | 80.0 | 0.95 | +27.1 | 1.0666 | +6.2 | 50[3] (2) | 91.0 (3) |
| *Twelve hours after injection of glucose solution* | | | | | | | | | | | |
| Normal untreated | 14 | 6.2 (9) | >41[4] | >0.04[4] | 80.5 | 0.82 | −7.0 | 1.0596 (8) | −5.0 | 73 (7) | 98.0 (7) |
| Hypx. untreated | 6 | 6.9 | >78[4] | >0.04[4] | 74.8 | 0.65 | +23.2 | 1.0630 | +4.4 | [3] | 84.4 (3) |
| Hypx.-cortical hormone | 8 | 9.3 | 40 | 0.04 | 86.5 | 0.41 | 0.0 | 1.0558 | −7.9 | 81 | 95.8 |
| Normal 0.9% NaCl | 7 | 3.1 | | | | | −9.3 | | | | |

[1] Four dead before six hours.
[2] Calculated from last three significant figures.
[3] Most pressures unreadable.
[4] Maximal values.
Numbers in parentheses indicate number of animals on which the particular determinations were made.

one of the initial responses to the intraperitoneal administration of glucose was an outpouring of fluid to the peritoneal cavity, resulting in an augmentation of the volume of the injected fluid at 3 hours. At 6 hours, absorption of fluid had begun, but more fluid was still present than had been injected; at 12 hours the volume of the ascitic fluid was about two-thirds of that injected.

The volume of ascitic fluid of hypophysectomized rats was not at any stage significantly different from that of normal animals.

A markedly different response was seen in adrenalectomized rats, which show little or no increase in ascitic fluid volume at 3 hours (Remington, 1940), and in our experiments the injected fluid was reduced by 18 per cent at 6 hours.

Less absorption of ascitic fluid had occurred at 12 hours in those hypophysectomized animals receiving injections of cortical hormone than in untreated hypophysectomized animals. This result is difficult to understand in view of the excellent maintenance of these animals. Further, although these treated animals absorbed less fluid than did the untreated, they excreted more urine, as noted above.

The intact animals injected with 0.9 per cent NaCl absorbed injected peritoneal fluid faster than the controls. Thus it is unlikely that the slow absorption in the treated hypophysectomized groups is attributable to the saline present in the cortical extract.

*Glucose in ascitic fluid* (*table 1, column 2a, 2b*). The rates of glucose absorption were the same in normal and hypophysectomized animals. Glucose was rapidly absorbed during the first 3 hours and at a diminishing rate thereafter.

As others have found, the adrenalectomized rat absorbed glucose more rapidly than either normal or hypophysectomized rats, a fact correlated with the rapid reduction of ascitic volume.

*Chloride in ascitic fluid* (*table 1, column 3*). Both total chloride and chloride concentrations in the peritoneal fluid were essentially the same in normal and hypophysectomized animals at all of the stages measured. Adrenalectomized animals, with lower volumes of ascitic fluid, had lower total chloride in the ascitic fluid.

*Specific gravity of ascitic fluid* (*table 1, column 4*). Between the sixth and twelfth hours there was a reduction in the specific gravity of the ascitic fluid, indicating probably the reabsorption of protein. Hypophysectomized animals showed slightly lower values than normal ones at both stages. The hypophysectomized rats which received cortical hormones had definitely lower values for specific gravity of the ascitic fluid than other groups, but the total amounts of protein were the same, due to the greater ascitic volumes.

*Hemoglobin* (*table 1, column 5*). The starting level of hemoglobin (5.15 gm. per cent) was slightly higher in normal than in hypophysectomized rats (14.1 gm. per cent). This difference was presumably a reflection of the early stages of the anemia which follows hypophysectomy (Vollmer, *et al.*, 1939).

After glucose injections hemoglobin rose rapidly, indicating hemoconcentration in all groups, but it rose to much higher levels in hypophysectomized than in normal animals.

Twelve hours after glucose was given, normal animals rediluted their blood to sub-normal hemoglobin values, but at that time there

was only slight relief of the hemoconcentration in the untreated hypophysectomized rats. In hypophysectomized animals receiving cortical hormones, the hemoglobin values returned to normal levels at 12 hours.

*Specific gravity of the blood* (*table 1, column 6a, 6b*). Blood specific gravity, like hemoglobin, was initially higher in normal than in hypophysectomized animals.

After glucose injections the values rose in all series, again giving some measure of the hemoconcentration. In normal animals the percentage changes paralleled almost exactly the hemoglobin figures. In the untreated hypophysectomized rats, however, the rise in blood specific gravity was uniformly less than that in hemoglobin.

It is possible that, associated with the circulatory failure described below in the untreated hypophysectomized rats, there was a leakage of blood proteins which accounted for the discrepancy between specific gravity and hemoglobin measurements as indices of hemoconcentration, It will be recalled, however, that there was no abnormal amount of protein in the ascitic fluid in these animals.

The changes in the specific gravity of the blood in the hypophysectomized animals which were treated with cortical hormones were similar to those in normal animals.

*Blood pressure and the peripheral circulation* (*table 1, column 8*). The control level of blood pressure was lower in hypophysectomized than in normal rats, confirming the observations of Leathem and Drill (1943) and the previous unpublished observations in this laboratory of Posin, Schweizer and Gaunt.

At 3 hours after injections of glucose the blood pressure fell markedly in all groups, the fall being greater in hypophysectomized than in normal animals, and more critical in the former because of the lower initial values. Adrenalectomized animals showed the greatest reduction of all. Cortical hormones stayed the fall of pressure in the hypophysectomized animals to such an extent that the percentage change from initial levels was less than that of normal animals.

At 6 hours, the blood pressure of the intact animals began to rise, but in most of the untreated hypophysectomized and adrenalectomized animals it presumably fell still further; collapse of peripheral circulation, however, made quantitative determinations impossible.

At 12 hours the blood pressure was still below normal in intact animals, and could not be read in any of the untreated hypophysectomized animals. In hypophysectomized animals receiving cortical hormones, pressures were restored to normal levels.

By the sixth hour it became clear that a derangement in peripheral circulation had taken place in the untreated hypophysectomized and adrenalectomized animals. A complete stasis occurred in many of the blood vessels of the web of the foot, and a reduced rate of blood flow occurred in all of them. In many cases, when the sluggish flow was

stopped by the pressure cuff, its restoration did not follow immediately upon the release of pressure. This obviously prevented the accurate measurement of the low blood pressures by the method used here, and was the state referred to above when it was said that pressures could not be read.

A comparable failure of peripheral circulation was not seen in intact animals, nor in hypophysectomized animals receiving cortical hormones.

*Body temperature* (*table 1, column 7*). Rectal temperatures were lower in untreated hypophysectomized than in normal rats at the outset. Following the injections of glucose, the temperatures fell in all groups, but to a much greater extent in untreated hypophysectomized and adrenalectomized animals than in normal ones. The therapy with cortical hormones was highly effective in checking the fall in temperature in hypophysectomized animals.

*Postmortem observations.* In all groups of animals considerable hyperemia of the intestine and mesentery was apparent. In many cases, especially in the untreated hypophysectomized and adrenalectomized rats, the ascitic fluid was contaminated with blood, and bleeding into the gut—a frequent concomitant of shock—had occurred.

## DISCUSSION

It is clear from the data presented here, together with that previously given by Gaunt, Schweizer and Remington (1937) and Remington (1940, 1943), that certain clear-cut distinctions can be made between the responses of normal, hypophysectomized, and adrenalectomized rats to the intraperitoneal injection of glucose solutions. Hypophysectomized animals, in which we are particularly interested here, resemble adrenalectomized ones in certain respects and normal ones in others.

It would appear that death in both hypophysectomized and addrenalectomized animals following glucose injections is attributable to circulatory failure, or shock. The classical symptoms of shock observed were a fall in blood pressure, hemoconcentration, failure of peripheral circulation and reduced body temperature. These features of the syndrome developed in essentially similar fashion in both hypophysectomized and adrenalectomized animals, although perhaps somewhat more rapidly in the latter. This set of symptoms, indeed, was the clearly significant differential between normal and hypophysectomized rats.

Since these symptoms were relieved by administration of adrenal cortical hormones, it is concluded that the deficiencies in the hypophysectomized animals were due to atrophy and hypofunction of the adrenal cortices.

One characteristic of the response to intraperitoneal glucose ob-

served in adrenalectomized animals, however, was almost entirely absent in animals subjected to hypophysectomy. During the early stages after injection, in normal and hypophysectomized animals, the volume of injected fluid is markedly augmented. In the adrenalectomized animal, however, the flow of fluid is largely in the opposite direction, and any increase in the volume of ascitic fluid is slight and transient, if present at all. At periods when normal and hypophysectomized animals have considerably more ascitic fluid than was injected, those without adrenals will have less. Although the amounts of ascitic fluid differ, the concentrations of the constituents which we measured were the same in the various groups, indicating that tonicity is approximately the same.

In short, the adrenalectomized animal given intraperitoneal glucose manifests *a*) an abnormal fluid and electrolyte behavior, and *b*) circulatory failure, or shock. Only the second of these responses is seen in the hypophysectomized animals.

This might appear to be a quantitative phenomenon in which the adrenals of the hypophysectomized animals are able to elaborate enough hormone to maintain normal fluid shifts, but not to prevent shock. However, Remington (1943), using adrenal preparations in adrenalectomized rats given intraperitoneal glucose, found that cortical hormones would not prevent the abnormal fluid disposition in these animals, although they readily prevented those changes which can be directly ascribed to circulatory failure.

This difference between the response of adrenalectomized and hypophysectomized animals could theoretically be accounted for by the fact that the adrenal medullae are present in the latter but not in the former (cf. Remington, 1943). Injections of adrenalin-in-oil have, however, failed to alter the response in adrenalectomized rats (unpublished observations), although fully conclusive observations have yet to be made.

From a variety of experiments it is apparent that adrenal cortical hormone is essential for the prevention of circulatory failure following stress (Swingle, Parkins, Taylor and Hays, 1938; Swingle, Remington, Drill and Kleinberg, 1942). Our experiments afford another example of susceptibility to circulatory failure in the absence of normal adrenal cortical function, in this case the primary deficiency being of the pituitary adrenotropic hormone.

## SUMMARY

The effects of intraperitoneal injections of isotonic glucose solution were studied in 84 normal, 78 hypophysectomized, and 6 adrenalectomized male rats.

Hypophysectomized animals resembled intact ones in respect to *a*) the initial transfer of fluid, electrolyte and protein to the peritoneal

cavity, and *b*), if the animals were not moribund, in their subsequent absorption of fluid, electrolyte, protein and glucose from the peritoneal cavity.

Hypophysectomized animals differed from normal ones in these respects: *a*) hemoconcentration was more severe, *b*) there was little or no hemodilution as ascitic fluid was absorbed, *c*) blood pressure and body temperature declined to lower levels, *d*) stasis occurred in the peripheral circulation (web of foot), *e*) little urine excretion occurred, and *f*) many animals succumbed in shock,.

All major deficiencies and death were prevented in hypophysectomized animals by the administration of adrenal cortical hormones, and therefore were presumably of adrenal cortical origin.

Hypophysectomized animals, while resembling adrenalectomized ones in susceptibility to the shock-like circulatory failure, did not, however, show similar shifts in water and electrolyte. Adrenalectomized animals transferred much smaller amounts of water and electrolyte to the peritoneal cavity in the early post-injection stages than either intact or hypophysectomized animals.

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# EFFECTS OF PITUITARY GONADOTROPINS ON ESTRUAL PHENOMENA IN EWES[1,2]

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ESTRUAL behavior has not been produced consistently in ewes by means of gonadotropic materials. Few or none of the anestrual ewes given a single injection of pregnant mares' serum by Cole and Miller (1933), McKenzie and Terrill (1937), Bell, *et al.*, (1941), and Hammond, *et al.*, (1942) showed behavioral estrus. Likewise, Bell, *et al.*, failed to obtain estrual behavior following a single series of injections of unfractionated pituitary extract. Cole and Miller (1933) found, however, that a high percentage of ewes came into heat when given a second injection 16 days later, and several other workers including Hammond, *et al.* (1942) have been able to confirm their observation. The latter workers went so far as to postulate that the presence of a degenerating corpus luteum, and presumably its waning progestin secretion, was necessary before gonadotropic hormone injections would produce estrus. They tested the hypothesis by giving injections of progesterone before injecting gonadotropic hormone, but the continued absence of heat failed to give supporting evidence for the hypothesis.

The present study includes work on the effects of pituitary gonadotropins, particularly those of a follicle-stimulating nature, upon the occurrence of estrus when administered to ewes in three different reproductive stages.

## EXPERIMENTAL ANIMALS AND PROCEDURE

The experiments were made during the winter and spring of 1940–1941 and of 1941–1942. The experimental animals of the first season were grade native ewes of various ages and predominantly of Hampshire and Shropshire breeding. Aged Western sheep from Montana were used the second season.

The occurrence of estrus was determined twice daily by the use of aproned teaser rams, and a ewe was considered to be in heat only if she stood for the ram to mount and remained standing after he had mounted.

The ewes received daily injections of follicle-stimulating extract[3] of sheep

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[3] Extracts prepared by Dr. W. H. McShan, Department of Zoology, University of Wisconsin. Follicle-stimulating extract prepared according to the method of McShan and Meyer (1940). Luteinizing and unfractionated extracts prepared by methods developed by McShan (unpub.).

pituitary powder over a 4-day period followed by an intravenous injection of luteinizing extract[2] (which was used in 1940–1941) or of unfractionated extract[3] (which was used in 1941–1942) 48 hours after the last injection of follicle-stimulating hormone. The total dosage of follicle-stimulating extract varied from 30 to 48 gram-equivalents per ewe for the different preparations. The intention, however, was to maintain the same total biological dosage on all animals. The average ovarian weight produced by 0.5 gram-equivalent of the different preparations in 21-day old Sprague-Dawley rats was 67 mg. The dosages of luteinizing and unfractionated extracts were kept constant at 10 and 1 gram-equivalent per ewe, respectively. Only qualitative assays were made with the luteinizing extract. One-tenth gram-equivalent of the unfractionated extract produced ovaries weighing approximately 75 mg. in 21-day old rats.

## EXPERIMENTAL RESULTS

*Ewes in the follicular phase.* A total of 35 ewes, for each of which there was detailed information on the length of at least two natural estrual cycles, were treated in what has been termed the 'follicular' phase of the estrual cycle, *i.e.*, treatment began the 12th day and

TABLE 1. COMPONENT PARTS OF ESTRUAL CYCLES BEFORE AND DURING TREATMENT WITH FOLLICLE-STIMULATING EXTRACT

| | Last 'Natural' Cycle, days | | 'Experimental' Cycle, days | |
|---|---|---|---|---|
| | Estrual period | Inter-estrual interval | Estrual period | Inter-estrual interval |
| 1940–1941 (18 ewes) | 1.53 | 15.61 | 2.30 | 14.83 |
| 1941–1942 (17 ewes) | 1.44 | 15.35 | 2.18 | 14.41 |
| Avg. for 2 seasons | 1.48 ± .06 | 15.49 ± .13 | 2.24 ± .10 | 14.63 ± .11 |

terminated the 17th day after the beginning of a heat period. Superovulation occurred in all these ewes.

The estrual periods which occurred during and immediately after treatment (experimental estrual periods) averaged 2.24 days in length which was a significant increase of 0.76 day or approximately 50 per cent of the length of the last natural estrual period of 1.48 days (table 1). The increased length of the estrual period came about as a result of the ewes coming into heat sooner and remaining in heat until about the time the heat period would normally have been expected to end. This is shown by the shortening of 0.86 day in the length of the inter-estrual interval. The lengths of time from the beginning of one heat period to the end of the next were approximately the same before and during treatment (18.46 and 18.36 days, respectively).

The yearly intra-ewe correlations of +.47 and +.42 (table 2) for all inter-estrual intervals previous to treatment show that ewes tend to have a characteristic length of inter-estrual interval. The repeat-

ability of the natural estrual periods (intra-ewe correlations of +.34 and +.15) is much less and is significant only in the first year. These observations are in accord with those of McKenzie and Terrill (1937) who found a distinct individuality in the length of the estrual cycle, but could not definitely establish individuality in the length of the estrual period.

The individuality of the cycle length for each ewe (correlation of +.59 between last natural and experimental intervals) was maintained during treatment in spite of the average shortening of the interestrual interval. Apparently the physiological mechanisms that

TABLE 2. RELATIONSHIPS OF ESTRUAL PERIOD AND INTER-ESTRUAL INTERVAL BEFORE AND DURING TREATMENT WITH FOLLICLE-STIMULATING EXTRACTS

| | 1940–1941 | 1941–1942 | Combined[1] |
|---|---|---|---|
| Intra-ewe correlation (natural inter-estrual intervals previous to treatment) | +.47[2] | +.42[2] | |
| Correlation between the last natural and the experimental inter-estrual interval | +.68[2] | +.48 | +.59[2] |
| Intra-ewe correlation (natural heat periods previous to treatment) | +.34[2] | +.15 | |
| Correlation between the last natural and the experimental estrual period | +.02 | +.14 | +.07 |

[1] Simple correlations combined by transforming to $Z$ as described by Fisher (1938).
[2] $P = < .01$.

operate in the ewe to bring about repeatability of the length of the natural inter-estrual interval also operate during treatment. Since the follicular phase of the estrual cycle was probably affected most by these treatments, it seems likely that the luteal phase is the one that is more highly characteristic of a given ewe.

The correlation of +.07 between the last natural estrual period and the experimental one provides no evidence that ewe individuality, if it exists, is maintained during treatment.

One of the possible effects from treatment being initiated on a fixed day of the estrual cycle might be to decrease the variation in the time of the beginning or of the end of the experimental heat. Although the interestrual intervals and therefore the estrual cycles were shortened, the variation in length of the estrual cycles was approximately the same with treatment as that observed in the last natural estrual cycle (table 3). Likewise, the variability in the time of end of heat, as indicated by the standard deviation of the length of time from the beginning of one estrual period to the end of the next, was not affected. Thus, there is no evidence that treatment during the follicular phase of the estrual cycle increases the predictability of the time when a ewe will come into or go out of heat.

*Ewes in the luteal phase.* During the 1941–1942 season, 5 ewes were treated in what has been termed the 'luteal' phase of the estrual cycle. Treatment began 3 days and terminated 9 days after the be-

ginning of a natural estrual period. The treatment induced ovulation in all the ewes, and one of them exhibited some symptoms of estrus at about the time of the intravenous injection. None of them, however, showed definite evidence of estrual behavior before they were slaughtered 3 days after the intravenous injection.

*Anestrual ewes.* In the spring of 1941, five ewes, and in the spring of 1942, four ewes, were treated after estrual cycles for the year had ceased. The only variation in the treatment as compared to that given other ewes was that 3 of the ewes in the second year received 5 rather than 4 subcutaneous injections of follicle-stimulating extract. One of

TABLE 3. VARIATION IN BEGINNING AND ENDING OF NATURAL AND EXPERIMENTAL ESTRUAL PERIODS

| | Standard deviation of estrual cycle length (beginning of one heat period to the beginning of the next) | Standard deviation of length of time from the beginning of one heat period to the end of the next |
|---|---|---|
| | *Last natural cycle* | |
| 1940–1941 | .61 | .57 |
| 1941–1942 | .69 | .63 |
| | *Experimental cycle* | |
| 1940–1941 | .59 | .62 |
| 1941–1942 | .49 | .62 |

the ewes in the first year was in heat when checked at 7 and again at 24 hours following the intravenous injection. One other ewe showed some signs of estrus when checked 7 hours after the intravenous injection, but was never definitely in heat. No other ewes showed evidence of estrual behavior, although ovulation occurred in all of them.

## DISCUSSION

Under the conditions of this study with the ewe, a follicle-stimulating extract of the pituitary gland caused a definite lengthening of the estrual period when administered in the latter part of the estrual cycle; it did not produce estrus when administered late in the first week of the estrual cycle, and brought about full estrual behavior in only 1 of 9 ewes treated during anestrus.

The increase of approximately 50 per cent in the length of the estrual period in ewes treated during the follicular phase of the estrual cycle can be interpreted as due to the attainment of the estrogen-threshold for estrual behavior at an earlier stage in the cycle when the ovaries are stimulated by gonadotropic extracts, than when estrus occurs naturally.

Ewes treated with follicle-stimulating extracts, however, during the first week of the estrual cycle or in anestrus failed, in most cases, to exhibit estrual behavior. The work of McKenzie and Terrill (1937) indicated that dosages of estradiol benzoate sufficient to produce

estrual behavior in a majority of anestrual and spayed ewes are not capable of bringing about estrus in ewes treated in midcycle. This makes it seem probable that in addition to estrogen production other factors, possibly associated with the activity of the corpus luteum, are involved in the estrual response, and makes any *a priori* assumption of a lack of estrogen production on the part of follicles stimulated during the luteal phase seem unwarranted.

The reason for the non-occurrence of heat in ewes treated during anestrus is not apparent. Several criteria including the discharge of mucus from the vagina of several of the ewes during treatment, the stimulated appearance of the uteri at the time of autopsy, and the unusual amount of interest shown in the ewes by the teaser rams, as well as the fact that one ewe came into heat, indicate that estrogen was being produced. Whether the typical absence of heat in these animals was due to too little or too great estrogenic activity or to the absence of some interacting factor (possibly a regressing corpus luteum) can be only a matter of speculation with the facts that are at hand.

## SUMMARY

Follicle-stimulating extracts of sheep pituitary powder administered subcutaneously and followed by luteinizing or unfractionated extracts intravenously during the last few days of the estrual cycle lengthened the estrual period an average of 0.76 day in 35 ewes. The natural variation in time of beginning and end of estrus was not affected. Individuality in the length of the inter-estrual interval was maintained.

None of 5 ewes treated similarly early in the estrual cycle and only one of 9 treated during anestrus showed full estrual behavior following treatment.

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# RELATION OF BODY WEIGHT TO LIVER GLYCOGEN STORAGE POTENCY OF ADRENAL CORTICAL EXTRACTS

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WE WISH to report certain observations that resulted from our experience with the method of Reinecke and Kendall (1942) proposed for the bio-assay of adrenal cortex extract based on the ability of the fasted adrenalectomized rat to store liver glycogen. Reinecke and Kendall chose to use rats weighing 140 to 160 gm. For convenience it seemed desirable to use animals of a much wider weight range. Accordingly a study was made of the effect of weight of rat on glycogen deposition. Our findings show that the response to a given dose of extract is independent of the body weight of the rat over a wide range of weight. Reinecke and Kendall used as a basis for comparison between a known and an unknown solution the amounts of glycogen expressed as mg. per 100 gm. of body weight. From our observations this is not a desirable method of expressing adrenal cortical glycogen activity.

## METHOD

The method of Reinecke and Kendall was adhered to essentially in the following details. Male albino rats were adrenalectomized and allowed 1 per cent sodium chloride solution and our stock diet[1] *ad libitum*. On the morning of the third day after adrenalectomy the food was removed. Extracts were injected subcutaneously 7 times at hourly intervals beginning the morning of the fourth day after adrenalectomy. Between 1 and 2 hours after the last injection of extract the animals were anesthetized with an intraperitoneal injection of 9 mg. of sodium amytal per 100 gm. of body weight. The entire liver was analyzed for glycogen by the method of Good, Kramer and Somogyi (1933). For these experiments the liver weight was also recorded so that comparisons could be based on milligrams of total glycogen, milligrams of glycogen per 100 gm. of body weight and per cent of glycogen. The glycogen values are expressed as glucose. The adrenal cortical extracts were prepared at The Wilson Laboratories.

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[1] The Wilson Laboratories rat stock diet contains the following pounds of ingredients per 100 pounds: 33 wheat meal, 32 corn meal, 21 milk powder, 7 linseed meal, 2 alfalfa meal, 2 liver meal, 1 Kelco meal, 1 cod liver oil, 0.5 bone meal and 0.5 iodized sodium chloride.

## RESULTS

Table 1 shows data which are typical of the results we have obtained by this method. The body weight included rats weighing 130 to 340 grams. For each dose of adrenal cortex extract, it will be seen that there was relatively no change in total content of liver glycogen regardless of the animal's weight. However, when the rats were compared on the basis of mg. of glycogen per 100 gm. of body weight, the values decreased as the weight of the rats increased. The rate of increase of the weight of the liver was not as rapid as the increase in body weight. Hence the values for percentage of glycogen decreased somewhat as body weight increased.

It has been the custom to calculate and express results on a single level of body weight such as 100 gm. with the idea that uniformity is

TABLE 1. LIVER GLYCOGEN DEPOSITION BY ADRENAL CORTICAL EXTRACTS IN RELATION TO BODY AND LIVER WEIGHT

| Experiment | Extract | Dose per Hour cc. | No. of Rats | Body Weight[1] gm. | Liver Weight gm. | Total Liver Glycogen mg.[3] | Glycogen mg. per 100 gm. Rat | Glycogen gm. per 100 gm. Liver |
|---|---|---|---|---|---|---|---|---|
| 10-26G 1 | 867 | 0.3 | 5 | 133 ± 3.3[2] | 4.41 ± 0.12 | 26.8 ± 1.2 | 21.9 ± 1.2 | 0.62 ± 0.02 |
| 10-26G 2 | 867 | 0.3 | 5 | 156 ± 6.5 | 5.68 ± 0.51 | 19.8 ± 5.1 | 13.6 ± 3.6 | 0.35 ± 0.10 |
| 10-26G 3 | 867 | 0.3 | 5 | 181 ± 9.2 | 5.56 ± 0.53 | 22.7 ± 6.7 | 13.7 ± 4.5 | 0.40 ± 0.16 |
| 10-26G 4 | 867 | 0.3 | 5 | 253 ± 10.2 | 7.24 ± 0.50 | 20.0 ± 4.9 | 8.4 ± 2.2 | 0.28 ± 0.08 |
| 11- 9G 5 | 867 | 0.5 | 4 | 158 ± 7.1 | 5.38 ± 0.27 | 36.9 ± 8.0 | 25.9 ± 6.8 | 0.69 ± 0.46 |
| 11- 9G 7 | 867 | 0.5 | 4 | 253 ± 4.2 | 7.07 ± 0.65 | 42.1 ± 15.3 | 18.0 ± 6.9 | 0.59 ± 0.20 |
| 11- 9G 6 | 867 | 2.0 | 4 | 158 ± 6.6 | 5.56 ± 1.01 | 53.8 ± 23.2 | 36.2 ± 15.6 | 0.94 ± 0.35 |
| 11- 9G 8 | 867 | 2.0 | 4 | 250 ± 8.7 | 6.96 ± 0.41 | 68.4 ± 6.5 | 30.1 ± 3.5 | 0.99 ± 0.07 |
| 3-22G 3 | K8 | 0.2 | 5 | 148 ± 11.7 | 4.85 ± 0.69 | 31.4 ± 7.5 | 23.1 ± 5.3 | 0.66 ± 0.15 |
| 3-22G 4 | K8 | 0.2 | 4 | 332 ± 6.6 | 7.82 ± 0.30 | 31.2 ± 15.1 | 10.3 ± 5.1 | 0.40 ± 0.18 |

[1] At the time of adrenalectomy.
[2] Standard deviation of a single observation.
[3] As glucose.

thereby attained. The data presented here show that such an assumption does not hold when animals of a wide weight range are compared, although there is apparent uniformity when narrow ranges of weight are selected. Similar reasons apply to the rejection of the method of reporting per cent of glycogen stored in the liver as a basis of comparison of potency. In addition, expressing results on a percentage basis requires the weighing of the liver. In so far as the conditions of our experiments hold, it is not necessary to weigh the liver and the variables of body weight and liver weight are eliminated in evaluating the relation between different doses of adrenal cortex extracts.

The relative constancy of the amount of glycogen stored in the entire liver per given dose of adrenal cortex extract, irrespective of the actual weight of the liver or of the rat, would indicate that the influence of the extract on glycogen formation is not a direct action on the liver but rather one to provide materials for metabolism by the liver. Our results do not show the mechanism involved. It has been

suggested by Long and his co-workers (1940) that "one of the properties of the cortical hormone is a stimulation of protein catabolism." Perhaps a given dose of adrenal cortex extract determines a level of protein catabolism and furnishes the liver with a definite amount of body protein or protein catabolites from which glycogen is formed.

As will be reported in another paper, the technic of Reinecke and Kendall of determining glycogen storage in the fasted adrenalectomized rat is in our opinion suitable for detecting differences in activity of adrenal cortical extracts. However, the question of expressing the results in units remains unanswered and must await the availability of a suitable reference standard.

## SUMMARY

In fasted adrenalectomized male rats injected with adrenal cortex extracts, the total amount of glycogen stored by the liver for a given dose of extract is independent of the body weight, at least in the range of 130 to 340 gm. The total amount of glycogen deposited is likewise independent of the weight of the liver.

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# EFFECT OF CONTINUED ORAL ADMINISTRATION OF DIETHYLSTILBESTROL ON BLOOD PRESSURE, HEART RATE AND RESPIRATION OF ALBINO RATS

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In 1940 Grollman, Harrison and Williams published evidence which indicated that diethylstilbestrol is capable of producing hypertension in rats. Since this is of obvious clinical importance and contrary to the data obtained from acute experiments conducted in this laboratory and elsewhere (Emery, *et al.*, 1943; Loeser and Erbacher, 1939) the study presented in this article was deemed pertinent.

## MATERIALS AND METHODS

The rats used in this experiment were of inbred albino stock from the Wistar strain weighing between 185 and 250 gm. They were maintained in a constant temperature room ($26 \pm 2$°C.), fed Purina dog chow and provided with a constant supply of fresh water. An alkaline aqueous solution of diethylstilbestrol[1] (1 mg. per 1 cc.) was given by stomach tube according to the method of Matthews, *et al.* (1941). The solution was made by dissolving 25 mg. of crystalline diethylstilbestrol in 0.5 cc. normal NaOH solution and then diluting with distilled water to a concentration of 1.0 mg. per cc.

A total of 45 castrated female rats and 35 normal male rats received 1 mg. of stilbestrol daily. The experimental groups were treated for varying periods up to 100 days. Comparable groups of untreated rats served as controls.

Both the direct and indirect method of blood pressure determination was utilized. In the case of the direct method the rat was lightly anesthetized with nembutal. A long abdominal incision was made and the abdominal aorta exposed from the bifurcation of the common iliac arteries to the point of origin of the renal vessels. A small glass canula was then inserted into the aorta, this being connected to a mercury manometer by means of a tube filled with sodium citrate solution (25%). These animals were a carefully selected group which showed no untoward signs resulting from the treatment and which sustained no undesirable effects from the operative procedure. The method selected for taking indirect blood pressure determinations was that of Williams, *et al.* (5). This procedure requires that the animal be heated but not anesthetized.

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[1] The authors wish to thank Dr. Erwin Schwenk of the Schering Corporation, Inc., Bloomfield, New Jersey, for the generous supply of diethylstilbestrol provided for this study.

## RESULTS

Figure 1 shows the individual blood pressure values obtained by the direct method after periods of treatment of from 4 to 100 days. The data are nearly equally distributed about our mean value for the control animals. Further, no general trend in either direction from

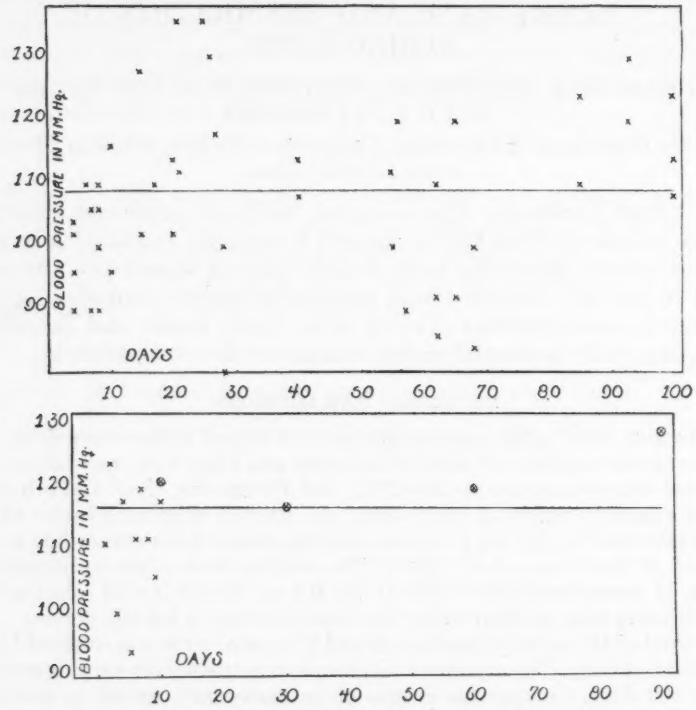


FIG. 1 (*upper*). Individual values of blood pressure taken by the direct method from 45 castrated female rats treated with stilbestrol for periods of 4–100 days. Solid line represents average blood pressure readings from 15 untreated control rats.

FIG. 2 (*lower*). Blood pressure values obtained by the indirect method from 35 male rats treated from 1–90 days with stilbestrol. Solid line represents average blood pressure of 16 untreated control rats. ×—represents mean pressure from a series of 10 rats treated daily for 8 days and recordings taken daily for 10 days. ⊗—represents series of 25 animals treated continuously for 90 days.

the normal is indicated at any time. The average value for all determinations was 107.0 mm. Hg for the treated group while that for the untreated group was 109.0 mm. (table 1). These values are so similar that further comment is superfluous. The range for both groups of animals is likewise quite similar, being 80 to 136 mm. and 84 to 128 mm. Hg for the treated and control groups, respectively (table 1).

The values secured for the heart rate and respiration were found to be 343 and 66 per minute, respectively, for the treated group

and 346 and 76.0, respectively, for the untreated animals.

The data obtained by the indirect method were taken from two series of animals. One group of 10 rats was treated daily for 8 days and blood pressure determinations made each day during the period of treatment. The medication was then discontinued and the blood pressure determined for an additional 4 days. It will be noted from figure 2 that no trend either to higher or lower levels is indicated during the 8 days of treatment. In addition no response was elicited upon cessation of treatment. Another group of 25 animals was continuously treated for a period of 90 days. Blood pressure determinations were made at 10, 30, 60, and 90-day intervals. The average values for these

TABLE 1. AVERAGE VALUES OBTAINED FROM 45 CASTRATED FEMALE RATS AND 15 UNTREATED CONTROL RATS

| | Weight, gm. | Blood Pressure mm. Hg | Range, B.P. mm. Hg | Heart Rate per mm. | Respiration per mm. |
|---|---|---|---|---|---|
| Treated | 213 | 107 | 80–136 | 343 | 66 |
| Controls | 214 | 109 | 84–128 | 346 | 76 |

periods were 121, 117, 120 and 129 mm. Hg, respectively, with an average for all groups of 122 mm. Hg which is comparable to the average for our control groups of 117 mm. Hg.

## DISCUSSION

A comparison of our values for the normal mean and range blood pressure with that of other workers is of considerable interest. Griffith and Farris (1942) using an indirect method for blood pressure determinations in rats anesthetized with nembutal obtained a range of from 60 to 120 mm. Hg with an average of 90 mm. and with ether, 80 to 140 mm. and an average of 110 mm. These values are comparable with our values obtained with the direct method and provide a check on our method and technic. The method of Williams, *et al.* (1939) which does not require anesthesia but necessitates heating the animal to approximately 40°C. gives somewhat higher values, their mean value for normal rats being 112.4 mm. Hg and the range 100 to 125 mm. Hg. Our mean value for the control group using this method was 117.0 mm. Hg.

Two methods were employed in an endeavor to exclude the undesirable effects of anesthesia and heat inherent in the procedures. While the indirect method consistently gave higher values than the direct method (the difference between those for the two treated and two control groups being 15 and 8 mm. Hg, respectively) these differences are not within hypertensive levels and since the values for both treated and control groups are so nearly similar it does not seem probable that any significance can be attached to this matter. Indeed this might well be expected since it represents the difference between the two unphysiologic conditions of anesthesia and heat. The good agree-

ment within each method indicates that the conditions of both procedures were well controlled.

Grollman, *et al.* (1940) in their study of the effect of diethylstilbestrol on blood pressure, treated 6 adult rats orally with 1 mg. per day for 8 days, determining blood pressure daily on the same rats by the method of Williams, *et al.* (1939). They obtained hypertensive levels of from 15 to 50 mm. The maximal values were reached on the tenth day with a subsequent decline. No values were given for periods of treatment longer than 8 days. These hypertensive levels are due, they believe, to renal injury. Our data not only indicate a normal range in blood pressure for the first 8 days of treatment but also reveal values within the normal range for subsequent readings taken at intervals during a period of treatment up to 90 days. In addition we secured readings at various intervals up to 3 months after cessation of treatment and these values were always found to be within the normal range.

The results obtained in this laboratory, therefore, differ from previous reports (Grollman, *et al.*, 1940; Leathem and Drill, 1943). The cause for this difference has not been revealed and we can only speculate as to its origin. Since we administered the sodium salt of diethylstilbestrol orally it would seem that some difference might be present in the drug in this form or that it undergoes an alteration during absorption from the digestive tract. In another group of 10 rats intramuscular injections of stilbestrol in peanut oil in 10 daily doses of 1 mg. each were given. In one rat the blood pressure was 120, 150, and 140 mm. Hg at the beginning, on the seventh and on the thirteenth days, respectively. The blood pressure of all of the other rats was well within the normal range at all times. Further work on this matter would be of interest.

## SUMMARY

Forty-five adult oöphorectomized rats and 35 normal male rats received by stomach tube 1 mg. of diethylstilbestrol in alkaline aqueous medium daily for periods up to 100 days. The results indicate no deviation of any kind from the normal range of values for the blood pressure. Neither was any trend toward higher values indicated for any period throughout the time of the experiment nor for a considerable time after treatment. No effect was detectable on the heart or respiratory rate.

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# FURTHER STUDY OF THE SPECIFICITY OF THE DIABETOGENIC EFFECT OF DIETHYLSTILBESTROL IN THE PARTIALLY DEPANCREATIZED RAT

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IT WAS first reported by Janes and Nelson (1940) that diethylstilbestrol has the property of increasing the glycogen content of liver in fasting normal rats. These same workers (1942) and also Long (1942) observed that the glycotropic effect of diethylstilbestrol in the rat was abolished by removal of either the pituitary or the adrenal glands, thereby supporting the hypothesis that the effect of diethylstilbestrol on carbohydrate metabolism is mediated by the adrenotropic activity of the anterior pituitary and the increased secretory activity of the adrenal cortex. Ingle (1941) found that diethylstilbestrol and other synthetic and naturally occurring estrogens exert a diabetogenic effect in partially depancreatized and in normal force-fed rats. This diabetogenic effect could not be duplicated by subjecting similar animals to conditions of stress which caused the adrenal cortices to hypertrophy. It was also shown by Ingle (1943; unpublished data) that diethylstilbestrol exerts a diabetogenic effect in partially depancreatized-adrenalectomized rats treated with sub-diabetogenic amounts of adrenal cortical extracts and that diethylstilbestrol will either induce or intensify a glycosuria in adrenalectomized-hypophysectomized-partially depancreatized rats maintained with sub-diabetogenic amounts of anterior pituitary and adrenal cortical extracts. The reasons for the discrepancy between the results obtained in studying the glycotropic effect and the diabetogenic effect of diethylstilbestrol are not clear.

Our interest in the mechanism of the effect of diethylstilbestrol on carbohydrate metabolism lies in the apparent similarity of its action to that of the adrenal steroids which also exert glycotropic and diabetogenic effects in the rat (Long, Katzin and Fry, 1940). It was the object of this study to evaluate further the specificity of the diabetogenic property of estrogens and adrenal steroids by testing other substances which might conceivably effect carbohydrate metabolism. Among the substances examined progesterone (Gaunt, Remington and Edelmann, 1939) has been found to increase the glycogen content of the liver in fasting ferrets and posterior pituitary extract (Neufeld and Collip, 1941) has been claimed to increase the glycogen content of the liver in fasting rats, mice, rabbits and guinea pigs. Calciferol and a preparation of pregnane derivatives extracted from the neutral fraction of pregnant mares' urine were tested because they each pos-

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sess a steroid ring system similar to the natural estrogens and adrenal steroids. An additional reason for testing calciferol is that it is one of the vitamin D compounds, some of which have been claimed to raise the blood sugar of the rabbit (Yamasaki, 1937) and to increase the liver glycogen of the rat (Pincussen, 1941). Phenol and the massive injection of water were tested as non-specific means of subjecting the animal to stress and thereby inducing hypertrophy of the adrenal cortices. It was found that these substances failed to manifest any diabetogenic activity under experimental conditions where diethylstilbestrol consistently exerted a diabetogenic effect.

## METHODS

Male rats of the Sprague-Dawley strain were used. Partial pancreatectomy was made at a body-weight of 200 to 300 gm. by removing all of the pancreas except that portion between the bile duct and the duodenum which was left intact in some rats and partially removed in others. The technique

TABLE 1. FLUID DIET USED IN FORCE-FEEDING OF RATS

| Constituent | Amount |
|---|---|
| Cellu flour (Chicago Dietetic Supply) | 120 gm. |
| Osborne & Mendel salt mixture | 40 gm. |
| Wheat germ oil | 10 cc. |
| Mazola oil | 175 cc. |
| Cod liver oil | 10 cc. |
| Dried yeast (Pabst) | 100 gm. |
| Whole milk powder (Merrell-Soule) | 600 gm. |
| Water to make total volume of | 2000 cc. |

previously described (Ingle and Griffiths, 1940) was modified according to Richter and Schmidt (1939) so that the pancreas lying between the duodenal loop was removed by suction through a small pipette.

Following partial pancreatectomy the rats were maintained on a diet of Purina dog chow until they had reached a weight of 300 to 375 gm. They were then placed in metabolism cages and maintained on a fluid diet administered by stomach tube each morning and late afternoon. The technique of force-feeding and the diets used were modifications of the methods described by Reinecke, Ball and Samuels (1939). The diet was made up according to table 1. During the period of adaptation to force-feeding the diet was first administered in small amounts and gradually increased until each rat was brought to a full feeding of 26 cc. per day on the 5th day. The available carbohydrate of the diet was approximately 5.0 gm. This value is based upon analyses of the diet and it is assumed that the maximum formation of glucose from protein does not exceed a glucose:nitrogen ratio of 3.6.

Progesterone and the pregnancy urine extract were dissolved in sesame oil and calciferol was ground into a fine suspension in sesame oil. The volume of oil was kept constant at 0.5 cc. per day. A water solution of posterior pituitary extract was used which had a concentration of 20 international units per cc. Phenol was made up in water solution and the volume was kept constant at 1.0 cc. per day. These preparations were all administered subcutaneously each morning and late afternoon. The massive intraperitoneal

injection of water was also made each morning and late afternoon. The diethylstilbestrol was dissolved in sesame oil (1.0 mg. per cc.) and doses of 0.1 mg. were administered once daily. Twenty-four-hour specimens of urine were collected at the same time each day and preserved with thymol. The determination of urinary glucose was made according to the method of Benedict (1911).

## EXPERIMENTS AND RESULTS

Experiment 1 was a study of progesterone, calciferol, posterior pituitary extract, phenol and massive intraperitoneal injections of

TABLE 2. GLYCOSURIA OF PARTIALLY DEPANCREATIZED RATS

| Rat No. | Daily Dose | Glycosuria mg./day[1] | | |
|---|---|---|---|---|
| | | Pre-inject | Experimental | Post-inject |
| | | *Progesterone, mg.* | | |
| 6 | 10 | 2270 | 2630 | 2815 |
| 106 | 10 | 1850 | 1315 | 2500 |
| 111 | 10 | 2500 | 2500 | 2750 |
| 13 | 10 | 3350 | 3550 | 2950 |
| 214 | 10 | 2270 | 2085 | 2500 |
| | | *Calciferol, mg.* | | |
| 106 | 1 | 3550 | 3500 | |
| | 5 | | 3330 | 3330 |
| 214 | 1 | 2500 | 2500 | 2775 |
| | | *Posterior pituitary* I. U. | | |
| 111 | 5 | 2750 | 2650 | 2500 |
| 315 | 20 | 1850 | 2000 | 2775 |
| | | *Water, cc., intraperitoneally* | | |
| 203 | 40 | 2500 | 2800 | |
| | 60 | | 2950 | 3570 |
| 214 | 40 | 2775 | 3330 | |
| | 60 | | 3250 | 3800 |
| | | *Phenol, mg.* | | |
| 413 | 100 | 1775 | 0 | 770 |
| 315 | 5 | 2775 | 2775 | |
| | 25 | | 3330 | |
| | 100 | | 4165 | 3850 |

[1] The highest single value for daily excretion occurring prior to injection, during the injection period, and following the injection period, are given here.

water for a possible effect on the amount of glucose excreted by partially depancreatized rats which had spontaneous glycosuria. All of the pancreas was removed except an approximate 50 per cent of the tissue lying between the bile duct and duodenum. The animals were observed during a control period until the extent of glycosuria became stable. The test substance was administered for a period of 7 days and was then either withdrawn or the dosage was increased for an additional period of 7 days. After the test substance was withdrawn the animals were observed during a second control period of 7 days. The data on the size of dose, number of animals tested and the highest values for urinary glucose observed during the first control period, the injection period and the second control period are sum-

marized in table 2. There was a tendency for the glycosuria to increase gradually in severity but when both the pre-injection and the post-injection control periods are considered it must be concluded that the test substances failed to exert any significant diabetogenic effect under the experimental conditions employed.

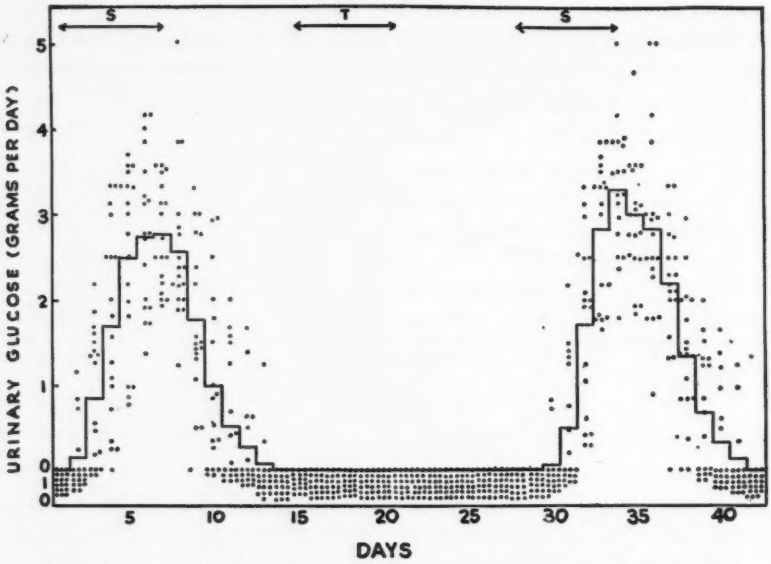


FIG. 1. Failure of certain test substances to induce glycosuria in partially depancreatized rats which were responsive to diethylstilbestrol. S = diethylstilbestrol; T = test substances.

The following substances were tested: progesterone, 6 rats at doses of 1, 2, and 5 mg. per day and 8 rats at the dose of 10 mg. per day; posterior pituitary extract, 1 rat at the dose of 1 I.U. per day, 4 rats at doses of 5, and 10 I.U. per day and 5 rats at the dose of 20 I.U. per day; calciferol, three rats at doses of 0.1, 0.5 and 1.0 mg. per day; and pregnancy urine extract, 2 rats at doses of 1 and 5 mg. per day.

Experiment 2 was a study of progesterone, calciferol, posterior pituitary extract, and the pregnancy urine extract for possible diabetogenic effect in partially depancreatized rats which did not have spontaneous glycosuria but were responsive to the diabetogenic activity of stilbestrol. In these test animals all of the pancreas was removed except for the tissue lying between the bile duct and duodenum The animals were observed during a minimum control period of 10 days after which they received a full feeding of diet (see methods). Diethylstilbestrol was administered for 7 days and then withdrawn. After a second control period of 7 days the test substance was administered for 7 days and was then either withdrawn or the dose was increased in amount for an additional period of 7 days. When the test substance was withdrawn the animals were observed during a third control period of 7 days. The substances tested, the size and number

of daily doses and the data on urinary glucose are summarized in figure 1. All of the rats were free of glycosuria during the control periods, all of them responded to diethylstilbestrol by developing glycosuria during the period of its injection and all of them failed to develop glycosuria when the other test substances were administered.

## DISCUSSION

The mechanism by which diethylstilbestrol and other estrogens exert a diabetogenic effect in the rat is not known. The hypothesis that the effect is mediated by the anterior pituitary and adrenal cortex does not provide an adequate explanation. In this study testing various other substances for diabetogenic activity in the rat, negative results were obtained. Of course, it is possible that other unrelated compounds not yet studied possess this property.

It does not seem profitable to examine other compounds unless some reason exists for believing that they may effect the metabolism of carbohydrate. Considerable importance is attached to the diabetogenic activity of certain adrenal steroids and physiologic interpretations are made of such data. It does not seem desirable to ignore the fact that diethylstilbestrol is very active in simulating some of the effects of adrenal steroids in the rat but is without benefit to adrenalectomized rats. A full explanation of the manner in which the cortical hormones influence the metabolism of carbohydrate and of its physiologic significance would require an understanding of the mechanisms by which corticomimetic compounds act.

## SUMMARY

Progesterone, calciferol, posterior pituitary extracts, phenol, a urine extract, and the massive intraperitoneal injection of water were tested for diabetogenic activity in partially depancreatized rats which consistently responded to diethylstilbestrol. The results were negative.

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# NOTES AND COMMENTS

## EFFECT OF TESTOSTERONE PROPIONATE ON THE PITUITARY GONADOTROPIC POTENCY OF THE CASTRATED MALE RAT[1]

HUMAN beings afflicted with primary gonadal failure excrete excessive amounts of gonadotropic hormone in the urine. The clinical picture of gonadal failure can be corrected by using small doses of the steroid hormones without causing depression of the excessive gonadotropic excretion (Heller and Heller, 1939; Heller and Myers, 1942; Heller, Farney, Morgan and Myers, 1942). Estrogen will not suppress the rise in gonadotropic potency of the pituitary gland of castrated (Lauson, Heller and Sevringhaus, 1938), unilaterally castrated or normal (Heller, Heller and Sevringhaus, 1942) female albino rats. The purpose of the present study was to determine the effect of testosterone propionate on the gonadotropic content of the pituitary glands of castrated male albino rats.

Thirty-eight male albino rats weighing 200 to 300 gm. were used. Thirty-two were castrated under ether anesthesia. Twenty-one days were allowed to elapse before hormone administration was begun, at which time the castrated rats were divided into 6 groups of 5 or 6 each. One group of 6 animals served as untreated castrated controls. Four groups were injected subcutaneously with, 1, 2, 5 and 10 mg. of testosterone propionate in 0.5 cc. of sesame oil, respectively. Subcutaneous injections were made thrice weekly for 3 weeks, the total elapsed time from castration to autopsy being 6 weeks.

The pooled anterior lobes were thoroughly macerated and suspended in sufficient tap water for each recipient assay rat to receive one gland equivalent. The final solution was injected twice daily, in 0.5 cc. quantities for 3 days. The immature albino female rats used in the assay were 22-days old on the day of the first injection. They were killed 24 hours after the last injection. The uterine and ovarian weights and the potency of the vaginal opening were recorded (Heller, Lauson and Sevringhaus, 1938).

The results are given in table 1. Typical castration changes occurred in the untreated castrated controls. These included a marked involution of the full and of the empty seminal vesicles, involution of the prostate gland, thymic hypertrophy, hypertrophy of the anterior pituitary gland, castration cell changes in the pituitary, and an increase in hypophyseal gonadotropic potency.

Even the 1 mg. dose of testosterone propionate caused marked hypertrophy response in the prostate glands and seminal vesicles. All doses caused

[1] This investigation was supported by a grant from Frederick Stearns and Company; administered by Dr. Richard M. Johnson.

thymic involution from the state of castration hypertrophy to weights below normal.

The 1 and 2 mg.-dose levels of testosterone propionate caused *a*) no significant reduction of anterior pituitary weight, *b*) no reversal of castration cell changes histologically and *c*), no depression of hypophyseal gonadotropic potency. The anterior pituitary glands of the rats treated with the 5 and 10 mg. dosages showed appreciable changes. The weights were lower, histo-

TABLE 1. EFFECT OF ORCHIDECTOMY AND ADMINISTRATION OF ANDROGENS UPON ORGAN WEIGHTS AND PITUITARY GONADOTROPIC POTENCY

| | Donor Rats, Gland Weights | | | | | | | Recipient Rats[2] | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of Animals | Seminal vesicles, full mg. | Seminal vesicles, empty mg. | Prostate mg. | Thymus mg. | Adrenal mg. | Anterior Pituitary mg. | No. of Animals | Ovarian wt. mg. |
| Untreated intact controls | 6 | 233 | 84 | 146 | 274 | 36 | 5.2 | 6 | 29.9 |
| Untreated castrated controls | 6 | 20 | 18 | 16 | 304 | 49 | 8.0 | 4 | 88.6 |
| Castrated animals treated with testosterone propionate[1] | | | | | | | | | |
| 1 mg. | 5 | 1005 | 275 | 524 | 98 | 46 | 7.9 | 4 | 98.1 |
| 2 mg. | 5 | 875 | 268 | 449 | 83 | 39 | 7.8 | 4 | 93.8 |
| 5 mg. | 5 | 1017 | 332 | 603 | 61 | 41 | 5.7 | 4 | 70.0 |
| 10 mg. | 5 | 891 | 312 | 555 | 72 | 42 | 5.5 | 4 | 48.9 |
| Pellets[1] | 6 | 858 | 267 | 550 | 69 | 39 | 6.1 | 5 | 66.0 |

[1] Injections were made 3 times weekly for 3 weeks. Dosage given is for single injections. An average of 34.9 mg. of free testosterone was absorbed from each pellet over the 3-week period.

[2] The ovarian weights of the recipient rats represent pituitary gonadotropic stimulation. Ovarian weights of uninjected recipients are 8–15 mg. Each recipient rat received one gland equivalent of a pooled suspension of donor pituitary glands.

logical examination showed reversal of castration changes, and there was a decrease of gonadotropic potency.[2] However, complete restoration to normal levels of the gonadotropic potency and of pituitary weight was not accomplished.

Androgen substitution in the castrated male rat did not restore the normal endocrine balance. This was concluded from the fact that the weights of the prostates and seminal vesicles were considerably above normal and the thymus glands showed atrophy, while restoration of gonadotropic potency and weight of the pituitaries to normal levels did not occur.

In general these findings are in agreement with those of Hamilton and Wolfe (1938) and Hellbaum and Greep (1943). The latter found suppression of the post-castration rise in gonadotropic potency when 0.3 mg. of testosterone propionate was given daily for 30 days, starting 5 days after orchidectomy. Larger doses failed to cause suppression when injections were begun 30 days following castration.

The effect of 75-mg. pellets of free testosterone[3] implanted subcutaneously was observed in the last group of castrated male rats. The pellets delivered

[2] The pituitary gonadotropic potency decrease is less when calculated as potency per milligram.

[3] The testosterone pellets and testosterone propionate were kindly supplied by Dr. Max Gilbert of the Schering Corp., Bloomfield, N. J.

34.9 mg., as judged by weights of pellets on removal, 21-days after implantation. The effect (table 1) upon reversal of castration changes was comparable to that produced by the 5.0 -mg. dose of testosterone propionate.

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